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<p>(21) International Application Number: PCT/US98/03271</p> <p>(22) International Filing Date: 19 February 1998 (19.02.98)</p> <p>(30) Priority Data: 08/801,344 19 February 1997 (19.02.97) US</p> <p>(71) Applicant: WISCONSIN ALUMNI RESEARCH FOUNDATION [US/US]; 614 Walnut Street, Madison, WI 53707-7365 (US).</p> <p>(72) Inventors: CAMERON, Douglas, C.; 610 Chatham Terrace, Madison, WI 53711 (US). SHAW, Anita, J.; 5B Waverly Court, Lansdale, PA 19446 (US). ALTARAS, Nedim, E.; 609 Constitution Lane, Madison, WI 53711 (US).</p> <p>(74) Agents: SARA, Charles, S. et al.; DeWitt Ross & Stevens S.C., 8000 Excelsior Drive, Madison, WI 53717-1914 (US).</p>	<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: MICROBIAL PRODUCTION OF 1,2-PROPANEDIOL FROM SUGAR</p>		
<pre> graph TD SUGARS --> DHAP SUGARS --> G3P[G-3-P] DHAP <--> tpi G3P DHAP --> methylglyoxal synthase MG G3P --> further metabolism MG --> glyoxalase I, glyoxalase II D_LACTATE[D-LACTATE] MG --> Recombinant Reductive Enzyme(s) 12PD[1,2-PD] subgraph Cell DHAP G3P MG D_LACTATE 12PD end style Cell fill:none,stroke:#000 </pre>		
<p>(57) Abstract</p> <p>Microorganisms which ferment common sugars into 1,2-propanediol, synthetic operons to effect the transformation, and methods to produce 1,2-propanediol by fermentation of common sugars using the transformed microorganisms are disclosed.</p>		

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MICROBIAL PRODUCTION OF 1,2-PROPANEDIOL FROM SUGAR

FIELD OF THE INVENTION

5 The invention is drawn to microorganisms and their use in the production of 1,2-propanediol via microbial fermentation of common sugars. More specifically, the present invention is drawn to recombinant microorganisms having reductive enzyme activity or activities which enable the recombinant microorganism to ferment common sugars to 1,2-propanediol.

BIBLIOGRAPHY

10 Complete bibliographic citations to the references mentioned below are included in the Bibliography section, immediately preceding the Abstract of the Disclosure. Each of the references mentioned below is incorporated herein by reference in its entirety.

DESCRIPTION OF THE PRIOR ART

15 1,2-Propanediol (1,2-PD; also known as propylene glycol) is a major commodity chemical with an annual production greater than one billion pounds in the United States. The major utilization of 1,2-PD is in unsaturated polyester resins, liquid laundry detergents, pharmaceuticals, cosmetics, antifreeze and de-icing formulations.

20 1,2-PD is conventionally produced from petrochemicals. Unfortunately, several toxic chemicals, such as chlorine, propylene oxide, and propylene chlorohydrin are either required or are produced as by-products in the conventional synthesis. In the

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conventional route, 1,2-PD is produced by the hydration of propylene oxide, which is obtained from propylene. The synthetic process produces racemic 1,2-PD, an equimolar mixture of the two enantiomers. This chemical process has a number of disadvantages, including the use of large quantities of water to minimize the production of polyglycols. The major problem, however, with the conventional synthetic route to 1,2-PD arises in the production of its intermediate, propylene oxide.

Propylene oxide is manufactured by one of two standard commercial processes: the chlorohydrin process or the hydroperoxide process. The chlorohydrin process involves toxic chlorinated intermediates and the use of caustic or lime. Additionally, this process may result in air emissions of propylene chlorohydrin and chlorine. (*Franklin Associates, Ltd. (1994).*) The hydroperoxide process involves oxidation of propylene by an organic hydroperoxide and results in the stoichiometric co-production of either *tert*-butanol or 1-phenyl ethanol. This makes the economics of the production of propylene oxide *via* the hydroperoxide route directly related to the market for the co-produced byproducts. (*Gait (1973).*)

It is known that 1,2-PD is produced by several organisms when grown on exotic sugars. As early as 1937, the fermentation of L-rhamnose to 1,2-PD (later shown to be the S enantiomer) was described by *Kluyver and Schnellen (1937)*. In *E. coli* and a variety of other microorganisms, L-rhamnose and L-fucose are metabolized to L-lactaldehyde and dihydroxyacetone phosphate. (*Sawada and Takagi (1964)* and *Gholambor and Heath (1962)*, respectively.) Under aerobic conditions, L-lactaldehyde is oxidized in two steps to pyruvate (*Sridhara and Wu (1969)*). Under anaerobic conditions, however, L-lactaldehyde is reduced to S-1,2-PD by a nicotinamide adenine nucleotide (NAD)-linked 1,2-propanediol oxidoreductase (EC 1.1.1.77). The S-1,2-PD produced diffuses into the extra-cellular medium.

Although a variety of microorganisms, including *E. coli*, produce S-1,2-PD from 6-deoxyhexose sugars, *Obradors et al. (1988)*, this route is not commercially feasible because these sugars are extremely expensive. The least expensive of these 6-

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deoxyhexose sugars, L-rhamnose, currently sells for approximately \$325 per kilogram (Pfanstiehl Laboratories, Chicago, Illinois).

In the mid-1980's, organisms capable of fermenting common sugars, such as glucose and xylose, to R-1,2-PD were discovered. See, for instance, *Tran-Din and Gotschalk* (1985). *Clostridium sphenoides* produces R-1,2-PD via a methylglyoxal intermediate. In this pathway, dihydroxyacetone phosphate (DHAP) is converted to methylglyoxal (MG) by the action of methylglyoxal synthase. The MG is reduced stereospecifically to give D-lactaldehyde. The D-lactaldehyde is then further reduced to give R-1,2-PD. The commercial production of 1,2-PD by *C. sphenoides* is severely limited, however, by the fact it is only produced under phosphate limitation; it is both difficult and expensive to obtain commercial-grade medium components which are free of phosphate. Additionally, only low titers of 1,2-PD are achieved.

Thermoanaerobacterium thermosaccharolyticum HG-8 (formerly *Clostridium thermosaccharolyticum*, ATCC 31960) also produces R-1,2-PD via methylglyoxal. *Cameron and Cooney* (1986). As with *C. sphenoides*, DHAP is converted to MG. The MG is then reduced at the aldehyde group to yield acetol. The acetol is then further reduced at the ketone group to give R-1,2-PD. For both *C. sphenoides* and *T. thermosaccharolyticum* HG-8, the enzymes responsible for the production of 1,2-PD have not been identified or cloned.

SUMMARY OF THE INVENTION

The invention is directed to a method of producing 1,2-propanediol by fermentation of sugars. The method comprises culturing a microorganism which expresses one or more enzymes which catalyze production of 1,2-propanediol from intracellular methylglyoxal in a medium containing a sugar carbon source other than a 6-deoxyhexose sugar, whereby the sugar carbon source is metabolized into 1,2-propanediol. Preferably, the method utilizes a recombinant organism containing one or

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more recombinant genes whose encoded gene products catalyze the reduction of methylglyoxal to 1,2-propanediol.

5 More specifically, the invention is directed to a method of producing 1,2-propanediol by fermentation with recombinant *E. coli* or yeast which comprises culturing a recombinant *E. coli* or yeast in a medium containing a sugar carbon source selected from the group consisting of arabinose, fructose, galactose, glucose, lactose, maltose, sucrose, xylose, and combinations thereof. The recombinant *E. coli* or yeast includes one or more recombinant genes which encode enzymes selected from the group consisting of aldose reductase, glycerol dehydrogenase, or combinations thereof.

10 The invention is also drawn to a synthetic operon which enables the production of 1,2-propanediol in a microorganism transformed to contain the operon. The operon includes one or more genes whose encoded gene products catalyze the reduction of methylglyoxal to 1,2-PD and a promoter sequence operationally linked to the one or more genes.

15 In a preferred embodiment, the synthetic operon includes at least one promoter sequence, a gene selected from the group consisting of an aldose reductase gene, a glycerol dehydrogenase gene, and combinations thereof; and a gene selected from the group consisting of a methylglyoxal synthase gene, a pyridine nucleotide transferase gene, and combinations thereof, wherein the genes are operationally linked to the promoter.

20 The invention is also drawn to *E. coli* transformed to contain the synthetic operon.

In short, the present invention is drawn to the use of microorganisms, preferably recombinant *E. coli* or *S. cerevisiae*, which express reductive enzyme activity which enables them to produce 1,2-PD, presumably via a reductive pathway leading from methylglyoxal to acetol (or lactaldehyde) to 1,2-PD.

25 If a recombinant microorganism is utilized, the gene sequences encoding the reductive enzyme activity may reside on plasmids within the microorganism, or the gene sequences may be integrated into the chromosome. It is preferred that the recombinant gene sequences be integrated into the genome of the microorganism.

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The invention utilizes microorganisms which express enzymes which enable the production of 1,2-PD from the fermentation of common sugars. As used herein, the term "common sugars" refers to readily available sugars including, but not limited to, arabinose, fructose, galactose, glucose, lactose, maltose, sucrose, and xylose. Specifically excluded from the term "common sugars" are 6-deoxyhexose sugars such as rhamnose and fucose.

While not being limited to a particular cellular mode of action, it is thought that by properly manipulating enzyme activity, intracellular MG is enzymatically reduced to yield 1,2-PD, which is then secreted into the extracellular environment.

The production of MG in the host microorganism can also be simultaneously increased, thereby increasing the production of 1,2-PD. Methylglyoxal production can be maximized by fermenting under phosphate limitation or with the addition of cAMP, as well as by several other methods known to the art. Additionally, selection of suitable host cells, such as methylglyoxal over-producing host cells or mutants which steer metabolism toward the production of 1,2-PD rather than other metabolites, can be utilized.

The invention is also drawn to a synthetic operons for transforming a host cell. When incorporated into a host cell, the operon directs the transformed host to produce enzyme activity which converts MG to 1,2-PD and may optionally include genetic elements to increase MG production or to increase the reducing power of the cell. Preferably, the operon includes one or more genes which encode enzymes necessary for expression of aldose reductase activity or glycerol dehydrogenase activity and one or more genes for increased production of MG in the host cell. The operon further includes upstream and/or downstream regulatory elements to control the expression of the gene products(s).

The synthetic operon sequence can be incorporated into any number of suitable and well-characterized plasmid vectors for incorporation into prokaryotic or eukaryotic host cells.

A major advantage of the present invention is that microbial fermentation provides a clean and "environmentally friendly" synthetic route to 1,2-PD. The microbial process

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uses as a substrate a renewable sugar such as glucose or xylose (found in agricultural crops) or lactose (found in dairy industry wastes). Suitable sugars are also produced in commodity amounts from corn and sugar cane and from lignocellulosic biomass.

Also, the microbial process produces no toxic wastes. The byproducts of fermentation are carbon dioxide, alcohols, and organic acids, all of which can be purified as valuable co-products or used as animal feed.

Another distinct advantage of the invention is that it provides a unique route to 1,2-PD from common sugars, a cheap, renewable, and readily available resource.

A further advantage of the present invention is that microbial processes are straightforward to operate and do not involve high temperatures and pressures. Large fermentation facilities such as those used for the production of ethanol can be readily adapted to the production of 1,2-PD.

Another advantage of the invention is that while MG is toxic to cells, by promoting overexpression of recombinant reductase activities, the recombinant cells remain viable and vigorous under conditions that promote MG production. In other words, any potentially toxic excess of MG produced in the recombinant host cell is rapidly converted to 1,2-PD by the recombinant reductase activity (or activities). The 1,2-PD formed is then exported from the cell.

The maximum theoretical yield of 1,2-PD from sugars is favorable: up to 1.5 moles 1,2-PD per mole sugar. And, unlike n-butanol, 1,2-PD itself has very low toxicity to microorganisms. This allows for good cellular growth and viability at high final product titers. Cellular growth at 100 g/L 1,2-PD has been obtained.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic diagram showing the metabolic production of 1,2-PD according to the present invention.

Fig. 2 is a schematic diagram of a preferred repressible transformation vector for use in the present invention, pSE380.

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Fig. 3 is an HPLC elution profile of media from recombinant *E. coli* strain AG1 cells which express exogenous aldose reductase activity showing production of 1,2-PD.

Fig. 4 is an HPLC elution profile of a 1,2-PD standard.

Fig. 5 is an HPLC elution profile of media from wild-type *E. coli* showing no production of 1,2-PD.

Fig. 6 is a graph depicting inducible production of 1,2-PD from recombinant *E. coli* containing an operon for the production and regulation of aldose reductase according to the present invention. Aldose reductase production was induced by the addition of IPTG to the culture medium.

Fig. 7 is a graph depicting the inhibition of cell growth due to the presence of 1,2-PD and 1,3-PD. As shown in the graph, 1,2-PD does not result in complete inhibition of cell growth until the amount added to the culture media is approximately 120 g/L.

DETAILED DESCRIPTION OF THE INVENTION

Overview:

An abbreviated schematic diagram of standard sugar metabolism, as well as the pathway for 1,2-PD production according to the present invention, are shown in Fig. 1. In non-transformed *E. coli*, sugars are converted to dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G-3-P) by glycolytic enzymes common to most organisms. The G-3-P is converted to metabolic byproducts such as ethanol, acetate, and succinate, and is also used for further metabolism.

DHAP is the initial intermediate in the 1,2-PD pathway. DHAP is converted to MG by methylglyoxal synthase. In non-transformed cells, the MG is metabolized to D-lactate as indicated in Fig. 1.

E. coli does not make 1,2-PD from sugars that are readily available. By manipulating various metabolic pathways leading both to and from MG, a microorganism can be made to produce 1,2-PD. While not being limited to any particular mode of

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action, it is thought that this reductive conversion takes place in two steps: 1) reduction of MG to acetol or lactaldehyde; and 2) reduction of acetol or lactaldehyde to 1,2-PD. Both reductions can be accomplished by a single enzyme activity or a combination of enzyme activities.

5 The crux of the invention, therefore, is a method to produce 1,2-PD using microorganisms which express enzyme activities whereby the microorganisms convert MG into 1,2-PD. The 1,2-PD so formed may then be harvested from the cell media. The microorganisms can be genetically altered organisms, including mutants or other recombinant strains.

10 The first step of the process is to identify and/or obtain the DNA sequences which encode the desired enzymes and insert or over-express them in the microorganism. This can be accomplished by any means known to the art.

15 For recombinant microorganisms, the preferred enzymes for the production of 1,2-PD are aldose reductase, glycerol dehydrogenase, or both. The preferred form of the aldose reductase gene is rat lens aldose reductase. The preferred form of the glycerol dehydrogenase gene is *E. coli* glycerol dehydrogenase. (In wild-type *E. coli*, glycerol dehydrogenase is regulated to prevent its catalyzing the conversion of MG to 1,2-PD.)

20 It must be noted, however, that because the aldose reductase sequence is highly conserved, the source of the aldose reductase gene is not critical to the present invention. (See, for instance, *Sato et al.* (1995) and *Old et al.* (1990)). Likewise, the source of the glycerol dehydrogenase gene is not critical to the success of the present invention, so long as the gene product displays the required reductive activity. The invention can be successfully practiced with any gene sequence whose expressed gene product provides reductive activity for the conversion of MG to 1,2-PD.

25 The rat lens aldose reductase gene has been cloned and sequenced and is available from the U.S. National Institutes of Health or can be obtained as described in *Sato et al.* and *Old et al.*, *supra*. Other aldose reductase gene sequences are available from "GENBANK" and can be synthesized or sub-cloned using any of several well known methods. Likewise, genes for glycerol dehydrogenase activity are known
30 ("GENBANK").

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5 The gene which encodes the enzyme having the required activity is then incorporated into a suitable vector which is used to transform a suitable cellular host. The preferred vector is a plasmid vector. The preferred host is a bacterial host, most preferably *E. coli*, although yeast such as *S. cerevisiae* can be utilized with equal success.

10 Incorporation of the gene into a plasmid transformation or shuttle vector is accomplished by digesting the plasmid with suitable restriction endonucleases, followed by annealing the gene insert to the plasmid "sticky ends," and then ligating the construct with suitable ligation enzymes to re-circulize the plasmid. Each of these steps is well known to those skilled in the art and need not be described in detail here. (See, for instance, *Sambrook, Fritsch, and Maniatis* (1986), *Molecular Cloning, A Laboratory Manual, 2nd Ed.*, incorporated herein by reference for its teaching of vector construction and transformation.)

15 Once successfully transformed with the required gene(s), the recombinant microorganisms produce 1,2-PD from the fermentation of all common sugars, including arabinose, fructose, galactose, glucose, lactose, maltose, sucrose, and xylose. Additionally, it has been shown that fermentation conditions which increase the formation of MG result in increased titers of 1,2-PD.

20 For purposes of this invention, increased MG production in the host cell can be obtained using any method now known or developed in the future. In *E. coli*, methods to obtain increased MG production include, but are not limited to: culturing under low-phosphate (*Ferguson et al.* (1996)), culturing with cyclic AMP and pentoses such as xylose or arabinose (*Ackerman et al.* (1974)), increasing intracellular DHAP (*e.g.* by culturing a triose phosphate isomerase knockout mutant), increasing conversion of DHAP to MG (*e.g.* by over-expressing methylglyoxal synthase), and culturing under unregulated metabolism. (See, for instance, *Freedberg et al.* (1971) and *Kadner et al.* (1992).)

25 Similarly, by utilizing MG over-producing mutants as the host, or by over-expressing endogenous genes (or by introducing exogenous genes) which promote the production of MG, production of 1,2-PD from the transformed cells is maximized.

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Careful selection of mutant hosts can also be used to increase the yield of 1,2-PD. *E. coli* mutants, such as AA200 (a triose phosphate isomerase knockout mutant, *E. coli* Genetic Stock Center, New Haven, Connecticut, U.S.A.), can be used as host cells to increase the intracellular levels of MG, thereby increasing 1,2-PD production. Similarly, glyoxalase knockout mutants can also be used as host cells, thereby increasing the intracellular level of MG for conversion to 1,2-PD. Appropriate host selection (using other *E. coli* mutants) also allows the conditions under which 1,2-PD is produced to be varied, e.g., aerobic or anaerobic production, different sugars as a carbon source, etc. For example, when transformed to express exogenous aldose reductase, the *E. coli* strain AA200 noted above has been shown to convert many sugars, including galactose, lactose, and sucrose, into 1,2-PD under aerobic conditions. Analogous transformations can also be accomplished in other host organisms, such as yeast.

Isolation of the 1,2-PD formed from the cell medium can be accomplished by any means known in the separation art. The preferred method is to filter the culture medium to separate cells and cellular debris, and then to isolate the 1,2-PD from the medium by vacuum distillation. (See, for instance, *Simon et al.* (1987).) If so desired, the recombinant microorganisms may be completely lysed by any known means prior to isolation of the 1,2-PD.

***E. coli* Transformed with pKKARX:**

For purposes of brevity and clarity only, the following description is limited to a transformation construct containing an aldose reductase gene. The identical procedure can be followed to insert any gene sequence having the proper activity, such as glycerol dehydrogenase, into a host to thereby enable or maximize the production of 1,2-PD. Other enzymes which promote production of 1,2-PD include: carbonyl reductase (EC 1.1.1.84), glycerol dehydrogenase (EC 1.1.1.6, EC 1.1.1.156), aldehyde reductase (EC 1.1.1.2), methylglyoxal reductase (also known as 2-oxoaldehyde reductase and lactaldehyde dehydrogenase, EC 1.1.1.78), L-glycol dehydrogenase (EC 1.1.1.185), alcohol dehydrogenase EC 1.1.1.1, EC 1.1.1.2), 1,2-PD dehydrogenase, (lactaldehyde

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reductase, EC 1.1.1.55), and 1,2-PD oxidoreductase, (lactaldehyde reductase, EC 1.1.1.77).

Any *E. coli* strain can be transformed to contain the aldose reductase insert described herein. The preferred strain is *E. coli* AG1 (*F*⁻, *endA1*, *hsdR17*, {*kan*^r, *mk*⁺} *supE44*, *thi1*, *recA1*, *gyrA96* *relA1*, λ), available commercially from Stratagene Corporation (La Jolla, California). This strain was used as the host strain for 1,2-PD production in the Examples described below unless otherwise noted. The AA200 and K10 strains were obtained from the *E. coli* Genetic Stock Center (New Haven, Connecticut).

Similarly, any yeast strain can be transformed to contain the desired gene insert. *S. cerevisiae*, numerous strains of which are available from a host of commercial suppliers and the American Type Culture Collection, is preferred.

For transformation of bacteria, a plasmid vector containing the gene insert is preferred. Several suitable vectors are available commercially or can be obtained by methods well known to the art. A preferred expression vector is pKK233-2, available commercially from the Pharmacia Biotech (Piscataway, New Jersey). The sequence of the pKK233-2 vector is shown in SEQ. ID. NO: 1. Suitable restriction enzymes and T4 DNA ligase to manipulate the vector can be obtained from several international suppliers, including Promega Corporation, (Madison, Wisconsin) and New England Biolabs (Beverly, Massachusetts).

The nucleotide sequence of the preferred rat lens aldose reductase gene is shown in SEQ. ID. NO: 3. The amino acid sequence of the encoded aldose reductase enzyme is shown in SEQ. ID. NO: 4.

The aldose reductase gene is inserted into the pKK233-2 plasmid (SEQ. ID. NO: 1) following standard procedures. (This process is essentially identical to that described by Old *et al.* (1990).) The resulting construct is designated pKKARX. The starting pKK233-2 plasmid is designed for direct cloning of eukaryotic genes in *E. coli*. The plasmid contains the highly expressed *trc* promoter (17 base pair spacing between the *trp*-35 region and the *lac* UV5-10 region), the *lacZ* ribosome binding site, and an ATG

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initiation codon. To prevent unstable replication, the strong *rrnB* transcription terminator has been introduced downstream of the Multiple Cloning Site. Digestion with *NcoI* exposes the start codon for direct ligation and expression of foreign proteins. Eukaryotic gene fragments lacking a prokaryotic ribosome binding site and/or an ATG can be inserted in the correct reading frame by using one of several commercially available *NcoI* linkers. (Available, for instance, from Pharmacia Biotech, Piscataway, New Jersey). The *NcoI* recognition sequence, CCATGG, commonly occurs at the initiation codon of eukaryotic genes, allowing direct ligation to the vector.

E. coli can then be transformed using the pKKARX construct. All transformations described herein were performed by the calcium chloride method using standard and well-known methodologies. While the calcium chloride method is preferred, transformation can be accomplished with equal success using any of several conventional procedures, such as electroporation and the like.

Once transformed with pKKARX, wild-type *E. coli* host cells produce 1,2-PD from arabinose, glucose, and xylose. Analysis for production of 1,2-PD is performed as described in Example 1, below.

***E. coli* Transformed with pSEARX:**

Another aspect of the invention is to transform the host with an insert which includes inducible or repressible genetic elements. This allows the production of 1,2-PD to be switched on or off by addition of a suitable inducer or repressor.

The preferred construct, designated pSEARX, is constructed by digesting pKKARX (described above) and a commercially-available vector designated pSE380 (Invitrogen, La Jolla, California) with *NcoI* and *EcoRI*. The resulting fragments from *NcoI* and *EcoRI* digestion are then separated by agarose gel electrophoresis, and the aldose reductase gene and pSE380 vector purified using "GENECLEAN" (Bio 101 Inc., La Jolla, California) according to the manufacturer's instructions. The two fragments are then ligated and transformed into AG1 using standard procedures (*Sambrook et al., supra*).

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5 A schematic of the starting pSE380 plasmid is shown in Fig. 2. The pSE380 plasmid includes a strong *trc* promoter for high level transcription, as well as the *lacO* operator and *lacI^r* repressor gene (which allows transcriptional regulation in any *E. coli* strain). While the pSE380 starting plasmid is preferred, any construct containing an inducible or repressible promoter which can control the expression of gene sequences operationally linked to the promoter will function with equal success. In addition to the *trc* promoter, examples of well known promoters which can be utilized include *lac*, *tac*, and *phoA*. The nucleotide sequence of pSE380 is given in SEQ. ID. NO: 2.

10 Inducing a wild-type *E. coli* host transformed with pSEARX by adding IPTG to the media results in the production of 1,2-PD when the host is grown on arabinose, glucose, and xylose.

Mutant host selection to maximize utilizable substrates and 1,2-PD production:

15 Increased flexibility when producing 1,2-PD from transformed *E. coli* or yeast is afforded by selection of a suitable mutant host. For instance, when transformed with either pKKARX or pSEARX as described above, triose phosphate isomerase knockout mutant bacteria, such as *E. coli* strain AA200, produce 1,2-PD when fermented with any combination of arabinose, galactose, glucose, lactose, sucrose, and xylose. Triose phosphate isomerase catalyzes the interconversion of DHAP to G-3-P. (See Fig. 1.) By
20 utilizing a host mutant which lacks triose phosphate isomerase activity, the metabolic fate of DHAP is directed to the formation of MG, which is then converted by various reductive enzyme activities into 1,2-PD, thereby increasing 1,2-PD titers.

25 Likewise, 1,2-PD production can be maximized by utilizing other mutants lacking one or more enzymes which decrease intracellular pools of MG. For instance, the normal metabolic pathway to detoxify intracellular methylglyoxal utilizes glyoxalase I. Glyoxalase I catalyzes the conversion of MG to S-D-lactoylglutathione, which is subsequently converted to lactate by glyoxalase II. Consequently, when a host is transformed to express a recombinant enzyme having MG reducing activity, the MG-reducing enzyme competes with glyoxalase I for the available MG. By utilizing

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glyoxalase I knockout mutants, the intracellular pool of MG for conversion to 1,2-PD is increased, and the ultimate production of 1,2-PD is likewise increased.

Glyoxalase mutants can be constructed in *E. coli*, yeast, or any other suitable host, using standard techniques. Because several glyoxalase oligonucleotide sequences are known ("GENBANK"), the most straightforward route to obtain a glyoxalase mutant is to recombine a deletion into the chromosomal copy of the glyoxalase gene whereby glyoxalase activity is destroyed. An example of how this can be done is described in *Knob et al.* (1994).

Negative Controls

To provide a negative control for the pKKARX and pSEARX constructs, a plasmid designated pKKARX/*Pst*I was constructed by digesting pKKARX with *Pst*I and purifying the vector portion of the resulting digest. The vector was then self-ligated resulting in an approximately 1 kb deletion within the aldose reductase gene on the plasmid. AG1 cells transformed with pKKARX/*Pst*I show no aldose reductase activity or 1,2-PD production.

Yeast Hosts:

In an analogous fashion, yeast (as well as other cellular hosts) can be transformed to contain the aldose reductase gene (or any of the other genes listed above) and can be used to produce 1,2-PD by fermentation of common sugars.

In yeast, the aldose reductase gene is first inserted into an appropriate shuttle vector. In the preferred embodiment, an aldose reductase cassette is ligated into YpJ66 digested with *Eco*RI/*Kpn*I, thus replacing the *galK* cassette with an aldose reductase cassette between *Eco*RI and *Kpn*I. YpJ66 is constructed from YEp352, whose oligonucleotide sequence is shown in SEQ. ID. NO: 5., and can be constructed according to the method of *Hill et al.* (1986). In short, this is accomplished by inserting the CUP1 promoter, (*galK*) and CYC1 terminator sequence into the *Xba*I site of Yep352.

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Preferably, the vector is then transformed into YPH500 (ATCC 76626) (*leu⁻*, *trp⁻*, *ura⁻*, *lys⁻*, *ade⁻*, *his⁻*) by standard methods and fed the required amino acids for growth, except uracil, which is used as the marker to maintain the plasmid in yeast. In the same fashion as transformed *E. coli*, yeast transformed to contain the aldose reductase insert produce 1,2-PD in isolatable quantities when fermented on a wide variety of common sugars, including galactose, glucose, sucrose, fructose, and maltose.

Other genetically altered strains can produce 1,2-PD when cultured on other sugar carbon sources such as xylose and lactose.

Synthetic Operons for the Production of 1,2-PD:

Ideally, three criteria should be maximized in order to maximize production of 1,2-PD. These three criteria are: increased production of MG, increased production of enzymes to convert MG to 1,2-PD, and increased production of enzymes such as pyridine nucleotide transferase to increase the reducing power within the cell (and thereby favor the reduction of MG to 1,2-PD). In this embodiment of the invention, a methylglyoxal synthase gene for increasing production of MG, and/or an aldose reductase or glycerol dehydrogenase gene for converting MG to 1,2-PD, and/or a pyridine nucleotide transferase gene for increasing the reductive power of the host cell are operationally linked, in any order, under the control of one or more promoters, to yield a synthetic operon which maximizes the production of 1,2-PD in host microorganisms transformed with the operon.

The methylglyoxal synthase gene has been cloned and expressed in *E. coli* and is shown in SEQ. ID. NO: 6. The ATG initiation codon is underlined. (See also *Percy and Harrison (1996)*). Likewise, the pyridine nucleotide transferase gene, encoding subunits A and B, is also known and is shown in SEQ. ID. NO: 7. The amino acid sequences of the encoded A and B subunits of pyridine nucleotide transferase are shown in SEQ. ID. NO: 8 and SEQ. ID. NO: 9, respectively. The glycerol dehydrogenase gene has also been identified; its oligonucleotide sequence is shown in SEQ. ID. NO: 10. The glycerol dehydrogenase amino acid sequence is shown in SEQ. ID. NO: 11.

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To construct the synthetic operon according to the present invention, SEQ. ID. NO: 3 (aldose reductase), SEQ. ID. NO: 6 (methylglyoxal synthase), SEQ. ID. NO: 7 (pyridine nucleotide transferase) and/or SEQ. ID. NO: 10 (glycerol dehydrogenase) are operatively linked together in a 5' to 3' orientation. The order of the genes is not critical to the functionality of the operon, so long as each gene is operationally linked to its neighbor in a 5' to 3' orientation.

The gene sequences are inserted into a suitable plasmid host which includes one or more promoter sequences such that the promoter is operationally linked to the gene sequences and can function to promote or repress transcription of the genes. Suitable promoter sequences include any number of well known and widely used promoters such as *lac*, *trc*, *tac*, and *phoA*. For instance, pSE380 contains the *trc* promoter. A very large number of suitable transformation vectors containing the above-listed promoters are commercially available from several international suppliers.

The gene insert containing the functional genes is constructed by standard and well known means. In short, the individual gene inserts are digested with an appropriate restriction enzyme to yield complimentary "sticky ends," which are then annealed to one another and ligated with T4 ligase. The gene construct is then again digested to yield appropriate complimentary ends to be operationally inserted into a plasmid vector containing the promoter sequences. Many commercial plasmids contain a Multiple Cloning Site which allows any number of different restriction enzymes to be utilized to effect insertion of the construct into the plasmid vector. The vector is then used to transform a suitable host, as described above.

When transformed with the synthetic operon as described herein, the recombinant microorganism produces 1,2-PD in isolatable quantities.

The synthetic operon need not contain any or all of the above-noted genes. At a minimum, at least one gene encoding an enzyme to effect the reduction of MG to 1,2-PD must be present, such as the aldose reductase gene or the glycerol dehydrogenase gene or some other gene or genes. In addition, either or both of the methylglyoxal synthase and pyridine nucleotide transferase genes may be present. Additionally, the

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genes need not all be under the control of a single promoter. For purposes of flexibility, each individual gene can be placed under the control of a separate promoter.

5 Additionally, an alternative to utilizing a triose phosphate isomerase knockout mutant host strain is to place the triose phosphate isomerase gene under the control of a promoter sequence. This enables transcription of the gene to be switched on or off, depending upon the conditions present. To effect insertion of promoter sequence in operational orientation to the triose phosphate isomerase gene, standard recombinant genetic techniques are utilized. (Again, see *Sambrook, Fritsch, and Maniatis* (1986), *Molecular Cloning, A Laboratory Manual, 2nd Ed.*) The promoter of interest is placed
10 into a suitable vector, preferably a plasmid vector, which contains appropriate cloning sequences to enable operational insertion of the promoter sequence into the genome of the host organism. Successful incorporation of the plasmid is determined via antibiotic resistance and/or testing for induction (or repression) of triose phosphate isomerase. Such method are well known to those skilled in the art.

15 EXAMPLES

The following Examples are included solely for illustrative purposes to provide a more complete understanding of the invention. The Examples do not limit the scope
20 of the invention disclosed or claimed herein in any fashion.

EXAMPLE 1: Chromatographic Analysis of Culture Broth

Figs. 3 and 5 depict HPLC analyses of the culture broth of an *E. coli* strain AG1 transformed to express aldose reductase (using pKKARX) and a non-transformed culture
25 of the same strain, respectively. Figure 4 depicts an HPLC elution profile of a 1,2-PD standard solution. With reference to Fig. 3 and 5, the fermentations were performed under standard anaerobic conditions using 5 g/L glucose as carbon source. Media samples were centrifuged and filtered before analysis.

To generate the plots shown in Figs. 3 and 5, an organic acids column (Bio-Rad
30 "HPX87H", Hercules, California) was used to quantify 1,2-PD, ethanol, sugars, and

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organic acids under the following conditions: 50 μ L sample size, pH 2 (H_2SO_4);, 0.5 mL/min flow rate, and 40°C column temperature. Peaks were detected by a refractive index detector at 40°C.

5 The 1,2-PD peak from the organic acids column was further analyzed by injection onto a cation-exchange column (Waters "SUGAR-PAK II," Marlboro, Massachusetts). The 1,2-PD peak isolated from the fermentation broth elutes at exactly the same time as the 1,2-PD control. The secondary peak identifications were performed on the "SUGAR-PAK II" column under the following conditions: 50 μ L sample size, Milli-Q water mobile phase; 0.5 mL/min flow rate; and 90°C column temperature.

10 Additionally, analyses were performed in which the 1,2-PD peak from the organic acids column was collected and subjected to gas chromatographic (GC) analysis and mass spectrographic analysis. The GC peak co-eluted with the 1,2-PD standard. Mass spectrometry showed the same fragmentation pattern as the 1,2-PD standard. The fact that the same peak co-eluted with a 1,2-PD standard on 3 different columns (HPLC organic acids column, HPLC sugars column, and GC), with different methods of separation, as well as its fragmentation in mass spectrography, its identification as 1,2-PD is quite certain.

EXAMPLE 2: Production of 1,2-Propanediol from Various Common Sugars

20 In this Example, a triose phosphate isomerase mutant (*tpi*-), AA200, was transformed with pSEARX containing the gene for aldose reductase as described above. (This transformed cell line is designated AA200::pSEARX). The non-transformed AA200 mutant yields higher intracellular concentrations of methylglyoxal, the precursor to 1,2-PD, than the wild-type. (See *Hopper and Cooper (1972)*.) When transformed with pSEARX, the AA200::pSEARX cell line produced 1,2-PD from arabinose, galactose, glucose, lactose, sucrose, and xylose. The yield of 1,2-PD from AA200::pSEARX fermented with various sugars was as follows:

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Table 1

<u>SUGAR</u>	<u>TITER 1,2-PD, mg/L</u>
Galactose	66
Glucose	71
Lactose	6
Sucrose	7
Xylose	49

Fermentation was performed using standard anaerobic fermentation procedures using 10 g/L of the appropriate sugar. The fermentation was allowed to proceed for 24 hours prior to analysis for 1,2-PD.

EXAMPLE 3: Inducible production of 1,2-PD

In this Example, the results of which are depicted in Fig. 6, *E. coli* strain AG1 was transformed as described herein with the pSEARX plasmid containing the aldose reductase gene. The transformed cells were then cultured under standard anaerobic conditions on 5 g/L glucose with increasing levels of the promoter IPTG. The X-axis of Fig. 6 gives the concentration of IPTG in millimolarity. The right-hand Y-axis (■) reports the production of 1,2-PD in mg/L as a function of IPTG concentration. Likewise the left-hand Y-axis (●) reports the activity of aldose reductase in U/mg. As is clearly shown in Fig. 6, inducing the promoter leads to the production of 1,2-PD.

EXAMPLE 4: Inhibition of Cell Growth by 1,2-PD

Here, an Experiment was performed to determine at what level the presence 1,2-PD and 1,3-PD begin to have an adverse effect on *E. coli* cell growth. Anaerobic batch cultivations of *E. coli* were carried out in 10 mL culture tubes. Nine different batch cultivations, covering a range between 0 and 120 g/L of 1,2-PD (○) and and 1,3-propanediol (□) were carried out in triplicate. Using optical density measurements, the growth in each tube was monitored and the specific growth rate determined. The results are depicted in Fig. 7. The ratio μ/μ_0 has been plotted as a function of the concentration of 1,2-PD and 1,3-PD (I, g/L). The value of μ equals the specific growth rate determined for the corresponding concentration of 1,2 or 1,3-PD; the value of μ_0 equals

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the specific growth rate determined in the absence of any 1,2-PD or 1,3-PD. The error bars indicate the standard deviation between the triplicate experiments. As can be seen from Fig. 7, 1,2-PD does not cause complete inhibition of cell growth until a concentration of approximately 120 g/L is reached.

**EXAMPLE 5: Anaerobic Production of 1,2-PD Utilizing
Recombinant Glycerol Dehydrogenase Gene**

E. coli strain AG1 was transformed in standard fashion with pSE380 containing a gene for *E. coli* glycerol dehydrogenase. The plasmid, designated pNEA10, was constructed in standard fashion. The transformed cells were then cultured under strictly anaerobic conditions on 10 g/L glucose. The fermentation was allowed to proceed for 12 hours to allow cell growth prior to addition of IPTG. The fermentation was then allowed to proceed for an additional 24 hours prior to analysis for 1,2-PD. The results are shown in Table 2:

Table 2

plasmid	IPTG mM	1,2-PD Titer mg/L	Activity* U/mg
pSE380†	0.0	0	0.10
pNEA10	0.0	100	0.48
pNEA10	0.05	190	3.00
pNEA10	0.10	220	2.70
pNEA10	0.25	220	3.10

*measured using glycerol as a substrate

†control plasmid without glycerol dehydrogenase gene

**EXAMPLE 6: Production of 1,2-PD by Host Containing Recombinant
Glycerol Dehydrogenase Gene in Combination With Promoter**

E. coli strain AG1 was transformed as described in Example 5. The transformed cells were then cultured on 15 g/L glucose under anaerobic conditions. Prior to the fermentation, the media was not purged of oxygen. IPTG was added at the start of the

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fermentation. The fermentation was allowed to proceed for 36 hours prior to analysis for 1,2-PD. The results are depicted in Table 3:

Table 3

plasmid	IPTG	1,2-PD Titer	Activity*
—	<u>mM</u>	<u>mg/L</u>	<u>U/mg</u>
pSE380†	0.0	0	0.10
pNEA10	0.0	30	2.31
pNEA10	0.05	100	9.89

*measured using acetol as a substrate

†control plasmid without glycerol dehydrogenase gene

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Cameron, Douglas C.
Shaw, Anita J.
Altaras, Nedim E.
- (ii) TITLE OF INVENTION: MICROBIAL PRODUCTION OF
1,2-PROPANEDIOL FROM SUGAR
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: DeWitt Ross & Stevens S.C.
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 - (C) CITY: Madison
 - (D) STATE: WI
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 53717-1914
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Sara, Charles S.
 - (C) REFERENCE/DOCKET NUMBER: 09820.037
- (ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4593 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic).

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vector pRK232-2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4476 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vector pSB380

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GTGCACGAGT GGGTTACATC GAACTGGATC TCAACAGCG TAAGATCCTT GAGAGTTTC      1320

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GCCCCGAAGA ACGTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT GGC GCGGTAT 1380
 TATCCCGTGT TGACGCCGGG CAAGAGCAAC TCGGTCGCCG CATACTAT TCTCAGAATG 1440
 ACTTGGTTGA GTACTACCA GTCACAGAAA AGCATCTTAC GGATGGCATG ACAGTAAGAG 1500
 AATTATGCAG TGCTGCCATA ACCATGAGTG ATAACACTGC GGCCAACCTA CTCTGACAA 1560
 CGATCGGAGG ACCGAAGGAG CTAACCGCTT TTTTGACAA CATGGGGGAT CATGTAATC 1620
 GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC AAACGACGAG CGTGACACCA 1680
 CGATGCCTGT AGCAATGGCA ACAACGTTGC GCAAACCTATT AACTGGCGAA CTACTTACTC 1740
 TAGCTTCCCG GCAACAATTA ATAGACTGGA TGGAGGCGGA TAAAGTTGCA GGACCACTTC 1800
 TCGCTCGGC CCTTCCGGCT GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG 1860
 GGTCTCGCGG TATCATTGCA GCACTGGGGC CAGATGGTAA GCCCTCCCGT ATCGTAGTTA 1920
 TCTACACGAC GGGGAGTCAG GCAACTATGG ATGAACGAAA TAGACAGATC GCTGAGATAG 1980
 GTGCCTCACT GATTAAAGCAT TGGTAACTGT CAGACCAAGT TTACTCATAT ATACTTTAGA 2040
 TTGATTTAAA ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT TTTGATAATC 2100
 TCATGACCAA AATCCCTTAA CGTGAGTTT CGTCCACTG AGCGTCAGAC CCCGTAGAAA 2160
 AGATCAAAGG ATCTTCTTGA GATCCTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA 2220
 AAAAACCACC GCTACCAGCG GTGGTTTGT TCCCGGATCA AGAGCTACCA ACTCTTTTC 2280
 CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCTTCTA GTGTAGCCGT 2340
 AGTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC 2400
 TGTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC 2460
 GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA 2520
 GCTTGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG 2580
 CCACGCTTC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG 2640
 GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTGCGGT 2700
 TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT 2760
 GGAAAAACGC CAGCAACGCG GCCTTTTAC GGTTCCTGGC CTTTGTCTGG CCTTTTGCTC 2820
 ACATGTTCTT TCCTGCGTTA TCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT 2880
 GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG 2940
 CGGAAGAGCG CCGATGCGG TATTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA 3000
 TATGCTGCAC TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATACCTCC 3060
 GCTATCGCTA CGTGAATGGG TCATGGCTGC GCGCCGACAC CCGCCAACAC CCGCTGACGC 3120
 GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG 3180

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GAGCTGCATG TGTGAGAGGT TTTCCACCGTC ATCACCGAAA CGCGCGAGGC AGCAGATCAA	3240
TTCCGCGCGC AAGGCGAAGC GGCATGCATT TACGTTGACA CCATCGAATG GCGCAAAACC	3300
TTTCGCGGTA TGGCATGATA GCGCCCCGAA GAGAGTCAAT TCAGGGTGGT GAATGTGAAA	3360
CCAGTAACGT TATACGATGT CGCAGAGTAT GCCGGTGTCT CTTATCAGAC CGTTTCCCGC	3420
GTGGTGAACC AGGCCAGCCA CGTTTCTGCG AAAACGCGGG AAAAAGTGGG AGCGGCGATG	3480
GCGGAGCTGA ATTACATTCC CAACCGCGTG GCACAACAAC TGGCGGGCAA ACAGTCGTTG	3540
CTGATTGGCG TTGCCACCTC CAGTCTGGCC CTGCACGCGC CGTCGCAAT TGTGCGGGCG	3600
ATTAAATCTC GCGCCGATCA ACTGGGTGCC AGCGTGGTGG TGTGATGGT AGAACGAAGC	3660
GGCGTGAAG CCTGTAAAGC GCGGGTGCAC AATCTTCTCG CGCAACGCGT CAGTGGGCTG	3720
ATCATTAACT ATCCGCTGGA TGACCAGGAT GCCATTGCTG TGAAGCTGC CTGCACTAAT	3780
GTTCGCGCGT TATTTCTTGA TGTCTCTGAC CAGACACCCA TCAACAGTAT TATTTTCTCC	3840
CATGAAGACG GTACGCGACT GGGCGTGGAG CATCTGGTGC CATTGGGTCA CCAGCAAATC	3900
GCGCTGTTAG CGGGCCCAAT AAGTTCTGTC TCGGCGCGTC TCGTCTGGC TGGCTGGCAT	3960
AAATATCTCA CTCGCAATCA AATTCAGCCG ATAGCGGAAC GGAAGGCGA CTGGAGTGCC	4020
ATGTCCGGTT TTCAACAAAC CATGCAAATG CTGAATGAGG GCATCGTTCC CACTGCGATG	4080
CTGGTGGCCA ACGATCAGAT GCGCTGGGC GCAATGCGCG CCATTACCGA GTCCGGGCTG	4140
CGCGTTGGTG CGGATATCTC GGTAGTGGGA TACGACGATA CCGAAGACAG CTCATGTTAT	4200
ATCCCGCCGT TAACCACCAT CAACAGGAT TTTCGCCTGC TGGGGCAAAC CAGCGTGGAC	4260
CGCTTGCTGC AACTCTCTCA GGGCCAGGCG GTGAAGGGCA ATCAGCTGTT GCCCGTCTCA	4320
CTGGTGAAAA GAAAAACCAC CCTGGCGCCC AATACGCAA CCGCCTCTCC CCGCGCGTTG	4380
GCCGATTCAT TAATGCAGCT GGCACGACAG GTTTCCCGAC TGGAAAGCGG GCAGTGAGCG	4440
CAACGCAATT AATGTGAGTT AGCGCGAATT GATCTT	4476

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(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1337 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Rat lens aldose reductase gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

CTCTTGCCGG TCGTTGTGCG TAACITGCAG CAATCATGGC TAGCCATCTG GAACTCAACA      60
ACGGCACCAA GATGCCACCC CTGGGTCTGG GCACCTGGAA GTCTCCTCCT GGCCAGGTGA      120
CCGAGGCTGT GAAGGTTGCT ATCGACATGG GGTATCGCCA CATTGACTGC GCCCAGGTGT      180
ACCAGAATGA GAAGGAGGTG GGGGTGGCCC TCCAGGAGAA GCTCAAGGAG CAGGTGGTGA      240
AGCGCCAGGA TCTCTTCATT GTCAGCAAGC TGTGGTGCAC GTTCCACGAC CAGAGCATGG      300
TGAAAGGGGC CTGCCAGAAG ACGCTGAGCG ACCTGCAGCT GGACTACCTG GACCTCTACC      360
TTATTCACCTG GCCAACTGGC TTCAAGCCTG GGCCTGACTA TTTCCCCCTG GATGCATCGG      420
GAAACGTGAT TCCTAGTGAC ACCGATTTTG TGGACACTTG GACGGCTATG GAGCAACTAG      480
TGGATGAAGG TTTGGTAAAA GCAATCGGAG TCTCCAACCT CAACCTCTCT CAGATTGAGA      540
GGATCTTGAA CAAACCTGGC TTAAAGTATA AGCCTGCTGT TAACCAGATC GAGTGCCACC      600
CATACCTAAC TCAGGAGAAG CTGATTGAGT ACTGCCATTG CAAAGGCATC GTGGTGACTG      660
CATACAGTCC CCTTGGTTCT CTTGACAGGC CTTGGGCCAA GCCTGAGGAC CCTCTCTCC      720
TGGAGGATCC CAGGATCAAG GAAATTGCAG CCAAGTACAA TAAACTACA GCCCAGGTGC      780
TGATCCGGTT CCCCATCCAA AGGAACCTGG TCGTGATCCC CAAGTCTGTG ACACCAACAC      840
GTATTGCTGA GAACTTTAAG GTCTTTGACT TTGAGCTGAG CAATGAGGAC ATGGCCACTC      900
TACTCAGCTA CAACAGGAAC TGGAGGTTGT GCGCCTTGAT GAGCTGTGCC AAACACAAGG      960
ATTACCCCTT CCACGCAGAA GTCTGAAGCT GTGGTGGACG AATCCTGCTC CTCCCCAAGC     1020
GACTTAACAC ATGTTCTTTC TGCTCATCTT GCCCTTGCAA GTGTCCCTCT GCACTGGGTG     1080
GCACCTTGCA GACCAGATGG TGAGAGTTTG TTAGTTTGAC GTAGAATGTG GAGGGCAGTA     1140
CCAGTAGCTG AGGAGTTTCT TCGGCCTTTC TTGGTCTTCT TCCCACCTGG AGGACTTTAA     1200
CAGGATACC TTTTCCAAAC AAGAGAAAG CAAGATTTAT AGCCCAAGTC ATGCCACTAA     1260
CACTTAAATT TGAGTGCTTA GAACTCCAGT CCTATGGGGG TCAGACTTTT TGCCTCAAT      1320

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AAAACTGCT TTTGTCG

1337

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 316 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Rat lens aldose reductase

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Ala Ser His Leu Glu Leu Asn Asn Gly Thr Lys Met Pro Thr Leu
1           5           10           15
Gly Leu Gly Thr Trp Lys Ser Pro Pro Gly Gln Val Thr Glu Ala Val
20          25          30
Lys Val Ala Ile Asp Met Gly Tyr Arg His Ile Asp Cys Ala Gln Val
35          40          45
Tyr Gln Asn Glu Lys Glu Val Gly Val Ala Leu Gln Glu Lys Leu Lys
50          55          60
Glu Gln Val Val Lys Arg Gln Asp Leu Phe Ile Val Ser Lys Leu Trp
65          70          75          80
Cys Thr Phe His Asp Gln Ser Met Val Lys Gly Ala Cys Gln Lys Thr
85          90          95
Leu Ser Asp Leu Gln Leu Asp Tyr Leu Asp Leu Tyr Leu Ile His Trp
100         105         110
Pro Thr Gly Phe Lys Pro Gly Pro Asp Tyr Phe Pro Leu Asp Ala Ser
115         120         125
Gly Asn Val Ile Pro Ser Asp Thr Asp Phe Val Asp Thr Trp Thr Ala
130         135         140
Met Glu Gln Leu Val Asp Glu Gly Leu Val Lys Ala Ile Gly Val Ser
145         150         155         160
Asn Phe Asn Pro Leu Gln Ile Glu Arg Ile Leu Asn Lys Pro Gly Leu
165         170         175
Lys Tyr Lys Pro Ala Val Asn Gln Ile Glu Cys His Pro Tyr Leu Thr
180         185         190
Gln Glu Lys Leu Ile Glu Tyr Cys His Cys Lys Gly Ile Val Val Thr
195         200         205
Ala Tyr Ser Pro Leu Gly Ser Pro Asp Arg Pro Trp Ala Lys Pro Glu
210         215         220

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Asp Pro Ser Leu Leu Glu Asp Pro Arg Ile Lys Glu Ile Ala Ala Lys
225                230                235                240

Tyr Asn Lys Thr Thr Ala Gln Val Leu Ile Arg Phe Pro Ile Gln Arg
                245                250                255

Asn Leu Val Val Ile Pro Lys Ser Val Thr Pro Ala Arg Ile Ala Glu
                260                265                270

Asn Phe Lys Val Phe Asp Phe Glu Leu Ser Asn Glu Asp Met Ala Thr
                275                280                285

Leu Leu Ser Tyr Asn Arg Asn Trp Arg Val Cys Ala Leu Met Ser Cys
290                295                300

Ala Lys His Lys Asp Tyr Pro Phe His Ala Glu Val
305                310                315

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5181 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Yeast shuttle vector YEp352

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

ATGACCATGA TTACGAATTC GAGCTCGGTA CCGGGGGATC CTCTAGAGTC GACCTGCAGG      60
CATGCAAGCT TGGCACTGGC CGTCGTTTCA CAACGTCGTG ACTGGGAAAA CCCTGGCGTT      120
ACCCAACTTA ATCGCCTTGC AGCACATCCC CCCTTCGCCA GCTGGCGTAA TAGCGAAGAG      180
GCCCCGACCG ATCGCCCTTC CCAACAGTTG CGCAGCCTGA ATGGCGAATG GCGCCTGATG      240
CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTCACACC GCATAGGGTA ATAACTGATA      300
TAATTAAATT GAAGCTCTAA TTTGTGAGTT TAGTATACAT GCATTTACTT ATAATACAGT      360
TTTTTAGTTT TGCTGGCCGC ATCTTCTCAA ATATGCTTCC CAGCCTGCTT TTCTGTAACG      420
TTCACCCCTCT ACCTTAGCAT CCCTTCCCTT TGCAAATAGT CCTCTTCCAA CAATAATAAT      480
GTCAGATCCT GTAGAGACCA CATCATCCAC GGTCTATAC TGTGACCCA ATGCGTCTCC      540
CTTGTCATCT AAACCCACAC CGGGTGTGAT AATCAACCAA TCGTAACCTT CATCTCTTCC      600
ACCCATGTCT CTTTGAGCAA TAAAGCCGAT AACAAATCT TTGTCGCTCT TCGCAATGTC      660
AACAGTACCC TTAGTATATT CTCCAGTAGA TAGGGAGCCC TTGCATGACA ATTCTGCTAA      720
CATCAAAGG CCTCTAGGTT CCTTTGTTAC TTCTTCTGCC GCCTGCTTCA AACCGCTAAC      780

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AATACCTGGG CCCACCACAC CGTGTGCATT CGTAATGTCT GCCCATTCTG CTATTCTGTA 840
TACACCCGCA GAGTACTGCA ATTTGACTGT ATTACCAATG TCAGCAAATT TTCTGTCTTC 900
GAAGAGTAAA AAATGTACT TGGCGGATAA TGCCTTTAGC GGCTTAACTG TGCCCTCCAT 960
GGAAAAATCA GTCAAGATAT CCACATGTGT TTTTAGTAAA CAAATTTTGG GACCTAATGC 1020
TTCAACTAAC TCCAGTAATT CCTTGGTGGT ACGAACATCC AATGAAGCAC ACAAGTTTGT 1080
TTGCTTTTCG TGCATGATAT TAAATAGCTT GGCAGCAACA GGACTAGGAT GAGTAGCAGC 1140
ACGTTCCCTTA TATGTAGCTT TCGACATGAT TTATCTTCGT TTCGGTTTTT GTTCTGTGCA 1200
GTTGGGTAA GAATACTGGG CAATTTTCATG TTTCTTCAAC ACTACATATG CGTATATATA 1260
CCAATCTAAG TCTGTGCTCC TTCCTTCGTT CTTCTTCTG TTCGGAGATT ACCGAATCAA 1320
AAAAATTTCA AAGAAACCGA AATCAAAAAA AAGAATAAAA AAAAAATGAT GAATTGAAAA 1380
GCTCTTGTTA CCCATCATTG AATTTTGAAC ATCCGAACCT GGGAGTTTTT CCTGAAACAG 1440
ATAGTATATT TGAACCTGTA TAATAATATA TAGTCTAGCG CTTTACGGAA GACAATGTAT 1500
GTATTTCCGT TCCTGGAGAA ACTATTGCAT CTATTGCATA GGTAATCTTG CACGTCGCAT 1560
CCCCGGTTCA TTTCTGCGT TTCCATCTTG CACTTCAATA GCATATCTTT GTTAACGAAG 1620
CATCTGTGCT TCATTTTGTA GAACAAAAAT GCAACGCGAG AGCGCTAATT TTTCAAACAA 1680
AGAATCTGAG CTGCATTTT ACAGAACAGA AATGCAACGC GAAAGCGCTA TTTTACCAAC 1740
GAAGAATCTG TGCTTCATT TTTTAAACA AAAATGCAAC GCGAGAGCGC TAAATTTTCA 1800
AACAAAGAAT CTGAGCTGCA TTTTACAGA ACAGAAATGC AACGCGAGAG CGCTATTTTA 1860
CCAACAAAGA ATCTATACTT CTTTTTGT CTACAAAAAT GCATCCCGAG AGCGCTATTT 1920
TTCTAACAAA GCATCTTAGA TTACTTTTTT TCTCCTTTGT GCGCTCTATA ATGCAGTCTC 1980
TTGATAACTT TTTGCACTGT AGGTCCGTTA AGGTAGAAAG AAGGCTACTT TGGTGTCTAT 2040
TTTCTCTTCC ATAAAAAAG CCTGACTCCA CTTCCCGCGT TTACTGATTA CTAGCGAAGC 2100
TGCGGGTGCA TTTTTTCAAG ATAAAGGCAT CCCCATTAT ATTCTATACC GATGTGGATT 2160
GCGCATACTT TGTGAACAGA AAGTGATAGC GTTGATGATT CTTATTGGT CAGAAAATTA 2220
TGAACGGTTT CTTCTATTTT GTCTCTATAT ACTACGTATA GGAAATGTTT ACATTTTCGT 2280
ATTGTTTTCG ATTCACTCTA TGAATAGTTC TTAACAAT TTTTTGTCT AAAGAGTAAT 2340
ACTAGAGATA AACATAAAAA ATGTAGAGGT CGAGTTTAGA TGCAAGTTCA AGGAGCGAAA 2400
GGTGGATGGG TAGGTTATAT AGGGATATAG CACAGAGATA TATAGCAAAG AGATACTTTT 2460
GAGCAATGTT TGTGGAAGCG GTATTGCAA TATTTTAGTA GCTCGTTACA GTCCGGTGCG 2520
TTTTGGTTT TTTGAAAGTG CGTCTTCAGA GCGCTTTGG TTTCAAAAG CGCTCTGAAG 2580
TTCCTATACT TTCTAGCTAG AGAATAGGAA CTTCGGAATA GGAACCTCAA AGCGTTTCCG 2640

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AAAACGAGCG CTTCCGAAAA TGCAACGCGA GCTGCGCACA TACAGCTCAC TGTTACGTC 2700
GCACCTATAT CTGCGTGTGG CCTGTATATA TATATACATG AGAAGAACGG CATAGTGGCT 2760
GTTTATGCTT AAATGCGTTA TGGTGCACTC TCAGTACAAT CTGCTCTGAT GCCGCATAGT 2820
TAAGCCAGCC CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGCTGCTCC 2880
CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT 2940
CACCCTCATC ACCGAAACGC GCGAGACGAA AGGGCCTCGT GATACGCCCTA TTTTATAGG 3000
TTAATGTCAT GATAATAATG GTTTCCTAGA CGTCAGGTGG CACTTTTCGG GGAAATGTGC 3060
GCGGAACCCC TATTTGTTTA TTTTCTAAA TACATTCAA TATGTATCCG CTCATGAGAC 3120
AATAACCCGT ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCAACATT 3180
TCCGTGTGCG CTTATTCCC TTTTTCGGG CATTTCGCT TCCGTTTTT GTCACCCAG 3240
AAACGCTGGT GAAAGTAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTACATCG 3300
AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCCAGAGAA CGTTTTCCAA 3360
TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT GACGCCGGGC 3420
AAGAGCAACT CGGTCGCCGC ATACACTATT CTCAGAATGA CTTGTTGAG TACTCACCAG 3480
TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA 3540
CCATGAGTGA TAACACTGCG GCCAATTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC 3600
TAACCGCTTT TTGCACAAC ATGGGGGATC ATGTAACCTG CCTTGATCGT TGGGAACCGG 3660
AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA GCAATGGCAA 3720
CAACGTTGCG CAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA 3780
TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGGCTG 3840
GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTGCAG 3900
CACTGGGGCC AGATGGTAAG CCTCCCCTA TCGTAGTTAT CTACACGACG GGGAGTCAGG 3960
CACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG ATTAAGCATT 4020
GGTAACTGTC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCATTTTT 4080
AATTTAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAA ATCCCTTAAC 4140
GTGAGTTTTC GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTGAG 4200
ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAACAAA AAAACCACCG CTACCAGCGG 4260
TGGTTTGTG GCCGGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAAGT GGCTTCAGCA 4320
GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA 4380
ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCACTG GCTGCTGCCA 4440
GTGGCGATAA CTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC 4500

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AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA 4560
CCGAAGTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA 4620
AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCAGC AGGGAGCTTC 4680
CAGGGGGAAA CGCCTGGTAT TTTTATAGTC CTGTCGGGTT TCGCCACCTC TGAATTGAGC 4740
GTGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG 4800
CCTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTGCTCA CATGTTCTTT CCTGCGTTAT 4860
CCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC GCTCGCCGCA 4920
GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC CCAATACGCA 4980
AACCCTCTCT CCCCCTGGCT TGGCCGATTC AITAATCCAG CTGGCAGGAC AGGTTTCCCG 5040
ACTGGAAGC GGGCAGTGAG CGCAACGCAA TTAATGTGAG TTACCTCACT CATTAGGCAC 5100
CCCAGGCTTT ACACTTTATG CTCCGGCTC GTATGTTGTG TCGAATTGTG AGCGGATAAC 5160
AATTTCACAC AGGAAACAGC T 5181

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 506 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: E. coli methylglyoxal synthase gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

TAAGTGCTTA CAGTAATCTG TAGGAAAGTT AACTACGGAT GTACATTATG GAACTGACGA 60
CTCGCACTTT ACCTGCGCGG AAACATATTG CGCTGGTGGC ACACGATCAC TGCAAACAAA 120
TGCTGATGAG CTGGGTGGAA CGGCATCAAC CGTTACTGGA ACAACACGTA CTGTATGCAA 180
CAGGCACTAC CGGTAAGTTA ATTTCCCGCG CGACCGGCAT GAACGTCAAC GCGATGTTGA 240
GTGGCCCAAT GGGGGGTGAC CAGCAGGTTG GCGCATTGAT CTCAGAGGGG AAAATTGATG 300
TATTGATTTT CTTCTGGGAT CCACTAAATG CCGTGCCGCA CGATCCTGAC GTGAAAGCCT 360
TGCTGCGTCT GCGCAGCGTA TGGAAACATC CCGTCGCCAC CAACGTGGCA ACGGCAGACT 420
TCATAATCCA GTCGCCGAT TTCAACGAGC CGGTCGATAT TCTGATCCCC GATTATCAGC 480
GTTATCTCGC GGACCGTCTG AAGTAA 506

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(2) INFORMATION FOR SEQ ID NO:7:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3524 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Pyridine nucleotide transhydrogenase gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

CATAAAAATA ATCCTTCGCC TTGCGCAAC CAGGTA CTGG TATTGTTATT AACGAGAAAC      60
GTGGCTGATT ATTGCATTAA AACGGTGTA CTGTCTGCGT CATTTTTCAT ATCACATTCC      120
TTAAGCCAAT TTAAATCTG CTCAAATGAC CGTCTATGCT TAAAAACAG CCGTATCAGC      180
ATCATTACTA CTGAAGCAAC TGAATTGTAT AAGTTAATTT AATGTTAAGT AGTGATTTCGT      240
GCCGGGGCGA TGTCTCGTTT TACCCGACCG TCGAAGACAA TTATCAGTCT TTATCCGGCG      300
TTCTAAGGTG TTTATCCAC TATCACGGCT GAATCGTTAA TATTTTGCGA GTTCACGCCG      360
AAATACTGAT TTTTGGCGCT AGATCACAGG CATAATTTTC AGTACGTTAT AGGGCGTTTG      420
TTACTAATTT ATTTTAACGG AGTAACATTT AGCTCGTACA TGAGCAGCTT GTGTGGCTCC      480
TGACACAGGC AAACCATCAT CAATAAAACC GATGGAAGGG AATATCATGC GAATTGGCAT      540
ACCAAGAGAA CGGTAAACCA ATGAAACCCG TGTTCAGCA ACGCCAAAAA CAGTGAACA      600
GCTGCTGAAA CTGGGTTTTA CCGTCGCGGT AGAGACCGGC GCGGGTCAAC TGGCAAGTTT      660
TGACGATAAA GCGTTTGTGC AAGCGGGCGC TGAATTGTA GAAGGGAATA GCGTCTGGCA      720
GTCAGAGATC ATTCTGAAGG TCAATGCGCC GTTAGATGAT GAAATTGCGT TACTGAATCC      780
TGGGACAACG CTGGTGAGTT TTATCTGGCC TGCGCAGAAT CCGGAATTAA TGCAAAAAC      840
TGCGGAACGT AACGTGACCG TGATGGCGAT GGA CTCTGTG CCGCGTATCT CACGCGCACA      900
ATCGCTGGAC GCACTAAGCT CGATGGCGAA CATCGCCGGT TATCGCGCCA TTGTTGAAGC      960
GGCAGATGAA TTTGGGCGCT TCTTTACCGG GCAAATTACT GCGGCCGGGA AAGTGCCACC      1020
GGCAAAAGTG ATGGTGATTG GTGCGGGTGT TGCAGGTCTG GCCGCCATTG GCGCAGCAAA      1080
CAGTCTCGGC GCGATTGTGC GTGCATTGGA CACCCGCCCC GAAGTGAAAG AACAAAGTTCA      1140
AAGTATGGGC GCGGAATTCC TCGAGCTGGA TTTTAAAGAG GAAGCTGGCA GCGGCGATGG      1200
CTATGCCAAA GTGATGTCGG ACGCGTTCAT CAAAGCGGAA ATGGA ACTCT TTGCCGCCCA      1260
GGCAAAAGAG GTCGATATCA TTGTACCAC CGCGCTTATT CCAGGCAAC CAGCGCCGAA      1320
GCTAATTACC CGTGAAATGG TTGACTCCAT GAAGGCGGEC AGTGTGATTG TCGACCTGGC      1380

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AGCCCAAAAC	GGCGGCAACT	GTGAATACAC	CGTGCCGGGT	GAAATCTTCA	CTACGGAAAA	1440
TGGTGTCAAA	GTGATTGGTT	ATACCGATCT	TCCGGGCCGT	CTGCCGACGC	AATCCTCACA	1500
GCTTTACGGC	ACAAACCTCG	TTAATCTGCT	GAAACTGTTG	TGCAAAGAGA	AAGACGGCAA	1560
TATCACTGTT	GATTTTGATG	ATGTGGTGAT	TCGCGGCGTG	ACCGTGATCC	GTGCGGGCGA	1620
AATTACCTGG	.CCGGCACCGC	CGATTCAGGT	ATCAGCTCAG	CCGCAGGCGG	CACAAAAAGC	1680
GGCACCGGAA	GTGAAACTG	AGGAAAAATG	TACCTGCTCA	CCGTGGCGTA	AATACGCGTT	1740
GATGGCGCTG	GCAATCATTC	TTTTTGCGTG	GATGGCAAGC	GTTGCGCCGA	AAGAATTCCT	1800
TGGGCACTTC	ACCGTTTTCG	CGCTGGCCTG	CGTTGTGCGT	TATTACGTGG	TGTGGAATGT	1860
ATCGCACGCG	CTGCATACAC	CGTTGATGTC	GGTCACCAAC	GCGATTTTCAG	GGATTATTGT	1920
TGTCGGAGCA	CTGTTGCAGA	TTGGCCAGGG	CGGCTGGGTT	AGCTTCCTTA	GTTTTATCGC	1980
GGTGCTTATA	GCCAGCATT	ATATTTTCGG	TGGCTTCACC	GTGACTCAGC	GCATGCTGAA	2040
AATGTTCCGC	AAAAATTAAG	GGGTAACATA	TGTCTGGAGG	ATTAGTTACA	GCTGCATACA	2100
TTGTTGCCGC	GATCCTGTTT	ATCTTCAGTC	TGGCCGGTCT	TTGAAACAT	GAAACGTCTC	2160
GCCAGGGTAA	CAACTTCGGT	ATCGCCGGGA	TGGCGATTGC	GTTAATCGCA	ACCATTTTTG	2220
GACCGGATAC	GGGTAATGTT	GGCTGGATCT	TGCTGGCGAT	GGTCATTGGT	GGGGCAATTG	2280
GTATCCGTCT	GGCGAAGAAA	GTTGAAATGA	CCGAAATGCC	AGAACTGGTG	GCGATCCTGC	2340
ATAGCTTCGT	GGGTCTGGCG	GCAGTGCTGG	TTGGCTTTAA	CAGCTATCTG	CATCATGACG	2400
CGGGAATGGC	ACCGATTCTG	GTCAATATTC	ACCTGACGGA	AGTGTTCCCTC	GGTATCTTCA	2460
TCGGGGCGGT	AACGTTACCG	GGTTCGGTGG	TGGCGTTCGG	CAAACTGTGT	GGCAAGATTT	2520
CGTCTAAACC	ATTGATGCTG	CCAAACCGTC	ACAAAATGAA	CCTGGCGGCT	CTGGTCGTTT	2580
CCCTCCTGCT	GCTGATTGTA	TTGTTTCGCA	CGGACAGCGT	CGGCCTGCAA	GTGCTGGCAT	2640
TGCTGATAAT	GACCGCAATT	GCGCTGGTAT	TCGGCTGGCA	TTTAGTCCGC	TCCATCGGTG	2700
GTGCAGATAT	GCCAGTGGTG	GTGTGATGC	TGAATCGTA	CTCCGGCTGG	GCGGCTGCGG	2760
CTGCGGGCTT	TATGCTCAGC	AACGACCTGC	TGATTGTGAC	CGGTGCGCTG	GTCGGTTCTT	2820
CGGGGGCTAT	CCTTTCTTAC	ATTATGTGTA	AGGCGATGAA	CCGTTCCITT	ATCAGCGTTA	2880
TTGCGGGTGG	TTTCGGCACC	GACGGCTCTT	CTACTGGCGA	TGATCAGGAA	GTGGGTGAGC	2940
ACCGCGAAAT	CACCGCAGAA	GAGACAGCGG	AACTGCTGAA	AACTCCCAT	TCAGTGATCA	3000
TTACTCCGGG	GTACGGCATG	GCAGTCGCGC	AGGCGCAATA	TCCTGTCGCT	GAAATTACTG	3060
AGAAATTGCG	CGCTCGTGGT	ATTAATGTGC	GTTTCGGTAT	CCACCCGGTC	GCGGGGCGTT	3120
TGCTCGGACA	TATGAACGTA	TTGCTGGCTG	AAGCAAAAGT	ACCGTATGAC	ATCGTGCTGG	3180
AAATGGACGA	GATCAATGAT	GACTTTGCTG	ATACCGATAC	CGTACTGGTG	ATTGGTGCTA	3240

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ACGATACGGT TAACCCGGCG GCGCAGGATG ATCCGAAGAG TCCGATTGCT GGTATGCCTG 3300
 TGCTGGAAGT GTGGAAAGCG CAGAACGTGA TTGTCTTTAA ACGTTCGATG AACACTGGCT 3360
 ATGCTGGTGT GCAAAACCCG CTGTTCTTCA AGGAAAACAC CCACATGCTG TTTGGTGACG 3420
 CCAAAGCCAG CGTGGATGCA ATCCTGAAAG CTCTGTAACC CTCGACTCTG CTGAGGCCGT 3480
 CACTCTTTAT TGAGATCGCT TAACAGAACG GCGATGCGAC TCTA 3524

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 510 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Pyridine nucleotide transhydrogenase, subunit A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Arg Ile Gly Ile Pro Arg Glu Arg Leu Thr Asn Glu Thr Arg Val
 1 5 10 15
 Ala Ala Thr Pro Lys Thr Val Glu Gln Leu Leu Lys Leu Gly Phe Thr
 20 25 30
 Val Ala Val Glu Ser Gly Ala Gly Gln Leu Ala Ser Phe Asp Asp Lys
 35 40 45
 Ala Phe Val Gln Ala Gly Ala Glu Ile Val Glu Gly Asn Ser Val Trp
 50 55 60
 Gln Ser Glu Ile Ile Leu Lys Val Asn Ala Pro Leu Asp Asp Glu Ile
 65 70 75 80
 Ala Leu Leu Asn Pro Gly Thr Thr Leu Val Ser Phe Ile Trp Pro Ala
 85 90 95
 Gln Asn Pro Glu Leu Met Gln Lys Leu Ala Glu Arg Asn Val Thr Val
 100 105 110
 Met Ala Met Asp Ser Val Pro Arg Ile Ser Arg Ala Gln Ser Leu Asp
 115 120 125
 Ala Leu Ser Ser Met Ala Asn Ile Ala Gly Tyr Arg Ala Ile Val Glu
 130 135 140
 Ala Ala His Glu Phe Gly Arg Phe Phe Thr Gly Gln Ile Thr Ala Ala
 145 150 155 160
 Gly Lys Val Pro Pro Ala Lys Val Met Val Ile Gly Ala Gly Val Ala

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165										170					175				
Gly	Leu	Ala	Ala	Ile	Gly	Ala	Ala	Asn	Ser	Leu	Gly	Ala	Ile	Val	Arg				
			180					185					190						
Ala	Phe	Asp	Thr	Arg	Pro	Glu	Val	Lys	Glu	Gln	Val	Gln	Ser	Met	Gly				
		195					200					205							
Ala	Glu	Phe	Leu	Glu	Leu	Asp	Phe	Lys	Glu	Glu	Ala	Gly	Ser	Gly	Asp				
	210					215					220								
Gly	Tyr	Ala	Lys	Val	Met	Ser	Asp	Ala	Phe	Ile	Lys	Ala	Glu	Met	Glu				
	225				230					235					240				
Leu	Phe	Ala	Ala	Gln	Ala	Lys	Glu	Val	Asp	Ile	Ile	Val	Thr	Thr	Ala				
				245					250						255				
Leu	Ile	Pro	Gly	Lys	Pro	Ala	Pro	Lys	Leu	Ile	Thr	Arg	Glu	Met	Val				
			260					265						270					
Asp	Ser	Met	Lys	Ala	Gly	Ser	Val	Ile	Val	Asp	Leu	Ala	Ala	Gln	Asn				
		275					280					285							
Gly	Gly	Asn	Cys	Glu	Tyr	Thr	Val	Pro	Gly	Glu	Ile	Phe	Thr	Thr	Glu				
	290					295					300								
Asn	Gly	Val	Lys	Val	Ile	Gly	Tyr	Thr	Asp	Leu	Pro	Gly	Arg	Leu	Pro				
	305				310					315					320				
Thr	Gln	Ser	Ser	Gln	Leu	Tyr	Gly	Thr	Asn	Leu	Val	Asn	Leu	Leu	Lys				
				325					330					335					
Leu	Leu	Cys	Lys	Glu	Lys	Asp	Gly	Asn	Ile	Thr	Val	Asp	Phe	Asp	Asp				
			340					345					350						
Val	Val	Ile	Arg	Gly	Val	Thr	Val	Ile	Arg	Ala	Gly	Glu	Ile	Thr	Trp				
		355					360					365							
Pro	Ala	Pro	Pro	Ile	Gln	Val	Ser	Ala	Gln	Pro	Gln	Ala	Ala	Gln	Lys				
	370					375					380								
Ala	Ala	Pro	Glu	Val	Lys	Thr	Glu	Glu	Lys	Cys	Thr	Cys	Ser	Pro	Trp				
	385				390					395					400				
Arg	Lys	Tyr	Ala	Leu	Met	Ala	Leu	Ala	Ile	Ile	Leu	Phe	Gly	Trp	Met				
				405					410					415					
Ala	Ser	Val	Ala	Pro	Lys	Glu	Phe	Leu	Gly	His	Phe	Thr	Val	Phe	Ala				
			420					425					430						
Leu	Ala	Cys	Val	Val	Gly	Tyr	Tyr	Val	Val	Trp	Asn	Val	Ser	His	Ala				
		435					440					445							
Leu	His	Thr	Pro	Leu	Met	Ser	Val	Thr	Asn	Ala	Ile	Ser	Gly	Ile	Ile				
	450					455					460								
Val	Val	Gly	Ala	Leu	Leu	Gln	Ile	Gly	Gln	Gly	Gly	Trp	Val	Ser	Phe				
	465				470					475					480				
Leu	Ser	Phe	Ile	Ala	Val	Leu	Ile	Ala	Ser	Ile	Asn	Ile	Phe	Gly	Gly				
				485					490					495					

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Phe Thr Val Thr Gln Arg Met Leu Lys Met Phe Arg Lys Asn
 500 505 510

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 462 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Pyridine nucleotide transhydrogenase, subunit B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ser Gly Gly Leu Val Thr Ala Ala Tyr Ile Val Ala Ala Ile Leu
 1 5 10 15

Phe Ile Phe Ser Leu Ala Gly Leu Ser Lys His Glu Thr Ser Arg Gln
 20 25 30

Gly Asn Asn Phe Gly Ile Ala Gly Met Ala Ile Ala Leu Ile Ala Thr
 35 40 45

Ile Phe Gly Pro Asp Thr Gly Asn Val Gly Trp Ile Leu Leu Ala Met
 50 55 60

Val Ile Gly Gly Ala Ile Gly Ile Arg Leu Ala Lys Lys Val Glu Met
 65 70 75 80

Thr Glu Met Pro Glu Leu Val Ala Ile Leu His Ser Phe Val Gly Leu
 85 90 95

Ala Ala Val Leu Val Gly Phe Asn Ser Tyr Leu His His Asp Ala Gly
 100 105 110

Met Ala Pro Ile Leu Val Asn Ile His Leu Thr Glu Val Phe Leu Gly
 115 120 125

Ile Phe Ile Gly Ala Val Thr Phe Thr Gly Ser Val Val Ala Phe Gly
 130 135 140

Lys Leu Cys Gly Lys Ile Ser Ser Lys Pro Leu Met Leu Pro Asn Arg
 145 150 155 160

His Lys Met Asn Leu Ala Ala Leu Val Val Ser Phe Leu Leu Leu Ile
 165 170 175

Val Phe Val Arg Thr Asp Ser Val Gly Leu Gln Val Leu Ala Leu Leu
 180 185 190

Ile Met Thr Ala Ile Ala Leu Val Phe Gly Trp His Leu Val Ala Ser
 195 200 205

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Ile Gly Gly Ala Asp Met Pro Val Val Val Ser Met Leu Asn Ser Tyr
 210                215                220

Ser Gly Trp Ala Ala Ala Ala Gly Phe Met Leu Ser Asn Asp Leu
 225                230                235                240

Leu Ile Val Thr Gly Ala Leu Val Gly Ser Ser Gly Ala Ile Leu Ser
                245                250                255

Tyr Ile Met Cys Lys Ala Met Asn Arg Ser Phe Ile Ser Val Ile Ala
                260                265                270

Gly Gly Phe Gly Thr Asp Gly Ser Ser Thr Gly Asp Asp Gln Glu Val
 275                280                285

Gly Glu His Arg Glu Ile Thr Ala Glu Glu Thr Ala Glu Leu Leu Lys
 290                295                300

Asn Ser His Ser Val Ile Ile Thr Pro Gly Tyr Gly Met Ala Val Ala
 305                310                315                320

Gln Ala Gln Tyr Pro Val Ala Glu Ile Thr Glu Lys Leu Arg Ala Arg
                325                330                335

Gly Ile Asn Val Arg Phe Gly Ile His Pro Val Ala Gly Arg Leu Pro
                340                345                350

Gly His Met Asn Val Leu Leu Ala Glu Ala Lys Val Pro Tyr Asp Ile
                355                360                365

Val Leu Glu Met Asp Glu Ile Asn Asp Asp Phe Ala Asp Thr Asp Thr
 370                375                380

Val Leu Val Ile Gly Ala Asn Asp Thr Val Asn Pro Ala Ala Gln Asp
 385                390                395                400

Asp Pro Lys Ser Pro Ile Ala Gly Met Pro Val Leu Glu Val Trp Lys
                405                410                415

Ala Gln Asn Val Ile Val Phe Lys Arg Ser Met Asn Thr Gly Tyr Ala
                420                425                430

Gly Val Gln Asn Pro Leu Phe Phe Lys Glu Asn Thr His Met Leu Phe
                435                440                445

Gly Asp Ala Lys Ala Ser Val Asp Ala Ile Leu Lys Ala Leu
 450                455                460

```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1139 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

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(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Glycerol dehydrogenase gene
(B) STRAIN: E. coli

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

TACGGCGTAA ACCGTGATGA GTAGAGATTT CCTCGTTAAT ACCTGGCGTA ATAAGTTAGT      60
GGCCCATTTA TGTAGGTCCC GCGACTACAC TAATTAGCAG ACCCGCTTAT GGACTTCGGC      120
GACTCTTGCG ACCAATCACC ACCCACTGTT TAAACAAAAT CCAAACGAG TTAGGTGACA      180
GCTCTTTTCG AAATTTCTAC GACCAGACCA TCATCTTTAA CGCGGCAAC CGCCACTTAC      240
AAGCGTTTTC CTCATGCTGG CAGACGCACC GTAGCGCCTC TGACGCGTCA CACCGCGTTA      300
AGAGCCATAG CCACCGCCTT TTTGGGAGCT ATGACGGTTT CGTGACCGTG TAAAGTACCC      360
ACAAGGCCAT CGCTAGCGTG GCTGATAGCG GAGATGGCTA CGTGGCACGT CGCGTAACAG      420
ACAATAGATG TGGCTACTCC CACTCAAACCT GGCGATAGAC GACAACGGTT TATTGGGCTT      480
ATACCGTAA CAGCTGTGGT TTTAGCAGCG ACCGCGTGGA CGTGCAGACA ATCGCCGCCC      540
ATAGCCGCTA CGCGACCGTT GGACCAAACCT TCGCGCACGG ACGAGAGCAT CGCCGCGCTG      600
GTGGTACCGC CCGCCGTTCA CGTGGGTCCG ACGCGACCGT GACCGACTTG ACACGATGTT      660
GTGGGACGAC CTTCTTCCGC TTTTTCGCTA CGAACGACGG CTTGTCATGC ATCACTGAGG      720
CCGCGACCTC GCGCACTAAC TTCGCTTG TGATAAACTCG CCACAACCAA AACTTTCACC      780
ACCAGACGAC GCCGCGTGCG TCACGTATTG CCGGACTGGC GATAGGGCCT GCGCGTAGTG      840
ATAATAGTGC CACTTTTTC CCGTAAGCCA TCGGACTGCG TCGACCAAGA CCTTTTACGC      900
GGCCACCTCC TTTAGCTTTG GCATCGACGG GAATCGGTAC GCCATCCAAA CGTTATTGAG      960
AGCGAGTTGA CCTATAATTT CTTCTACAGG GCCCGTTTTA CGTTAACAC CGTCTTCGCC      1020
GTACACGCTT TCCACTTTGG TAAGTGTGTG ACGGACCGCC GCGCTGCGGT CTAGTCCAAA      1080
TGCGGCGAGA CGACCATCGG CTGGTCATGC CAGTCGCAAA GGACGTTCTC ACCCTTATT      1139

```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 380 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: E. coli glycerol dehydrogenase

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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Met Pro His Leu Ala Leu Leu Ile Ser Lys Gly Ala Ile Met Asp Arg
 1 5 10 15
 Ile Ile Gln Ser Pro Gly Lys Tyr Ile Gln Gly Ala Asp Val Ile Asn
 20 25 30
 Arg Leu Gly Glu Tyr Leu Lys Pro Leu Xaa Glu Arg Trp Leu Val Val
 35 40 45
 Gly Asp Lys Phe Val Leu Gly Phe Ala Gln Ser Thr Val Glu Lys Ser
 50 55 60
 Phe Lys Asp Ala Gly Leu Val Val Glu Ile Ala Pro Phe Gly Gly Glu
 65 70 75 80
 Cys Ser Gln Asn Glu Ile Asp Arg Leu Arg Gly Ile Ala Glu Thr Ala
 85 90 95
 Gln Cys Gly Ala Ile Leu Gly Ile Gly Gly Lys Thr Leu Asp Thr
 100 105 110
 Ala Lys Ala Leu Ala His Phe Met Gly Val Pro Val Ala Ile Ala Pro
 115 120 125
 Thr Ile Ala Ser Thr Asp Ala Pro Cys Ser Ala Leu Ser Val Ile Tyr
 130 135 140
 Thr Asp Glu Gly Glu Phe Asp Arg Tyr Leu Leu Leu Pro Asn Asn Pro
 145 150 155 160
 Asn Met Val Ile Val Asp Thr Lys Ile Val Ala Gly Ala Pro Ala Arg
 165 170 175
 Leu Leu Ala Ala Gly Ile Gly Asp Ala Leu Ala Thr Trp Phe Glu Ala
 180 185 190
 Arg Ala Cys Ser Arg Ser Gly Ala Thr Thr Met Ala Gly Gly Lys Cys
 195 200 205
 Thr Gln Ala Ala Leu Ala Leu Ala Glu Leu Cys Tyr Asn Thr Leu Leu
 210 215 220
 Glu Glu Gly Glu Lys Ala Met Leu Ala Ala Glu Gln His Val Val Thr
 225 230 235 240
 Pro Ala Leu Glu Arg Val Ile Glu Ala Asn Thr Tyr Leu Ser Gly Val
 245 250 255
 Gly Phe Glu Ser Gly Gly Leu Ala Ala Ala His Ala Val His Asn Gly
 260 265 270
 Leu Thr Ala Ile Pro Asp Ala His His Tyr Tyr His Gly Glu Lys Val
 275 280 285
 Ala Phe Gly Thr Leu Thr Gln Leu Val Leu Glu Asn Ala Pro Val Glu
 290 295 300
 Glu Ile Glu Thr Val Ala Ala Leu Ser His Ala Val Gly Leu Pro Ile
 305 310 315 320
 Thr Leu Ala Gln Leu Asp Ile Lys Glu Asp Val Pro Ala Lys Met Arg
 325 330 335

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Ile Val Ala Glu Ala Ala Cys Ala Glu Gly Glu Thr Ile His Asn Met
340 345 350

Pro Gly Gly Ala Thr Pro Asp Gln Val Tyr Ala Ala Leu Leu Val Ala
355 360 365

Asp Gln Tyr Gly Gln Arg Phe Leu Gln Glu Trp Glu
370 375 380

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CLAIMS

What is claimed is:

1. A method of producing 1,2-propanediol by fermentation of sugars comprising: culturing a recombinant microorganism which expresses one or more enzymes which catalyze production of 1,2-propanediol from intracellular methylglyoxal in a medium containing a sugar carbon source other than a 6-deoxyhexose sugar, whereby the sugar carbon source is metabolized by the microorganism into 1,2-propanediol.

2. The method of Claim 1, wherein a recombinant microorganism containing one or more recombinant genes whose encoded gene products catalyze the reduction of methylglyoxal to 1,2-propanediol is cultured.

3. The method of Claim 1 or Claim 2, wherein a recombinant *E. coli* is cultured.

4. The method of any one of Claims 1, 2, or 3, wherein a recombinant microorganism which expresses enzyme activity selected from the group consisting of recombinant aldose reductase activity, recombinant glycerol dehydrogenase activity, recombinant methylglyoxal synthase activity, recombinant pyridine nucleotide transferase, and combinations thereof, is cultured.

5. The method according to any one of the preceding claims, wherein a recombinant microorganism transformed with a transformation vector containing a gene sequence selected from the group consisting of SEQ. ID. NO: 3, SEQ. ID. NO: 6, SEQ. ID. NO: 7, SEQ. ID. NO: 10, and combinations thereof, is cultured.

6. The method according to any one of the preceding claims, wherein a recombinant microorganism transformed with a transformation vector containing a gene sequence selected from the group consisting of SEQ. ID. NO: 3, SEQ. ID. NO: 6, SEQ. ID. NO: 7, SEQ. ID. NO: 10, and combinations thereof, the gene sequence operationally linked to one or more promoter sequences whereby transcription of the gene sequence is controlled, is cultured.

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7. The method of Claim 6, wherein the promoter sequence is selected from the group consisting of *lac*, *trc*, *tac*, and *phoA*.

8. The method according to any one of the preceding claims, wherein a microorganism lacking enzyme activity selected from the group consisting of triose phosphate isomerase activity, glyoxalase I activity, and combinations thereof, is cultured.

9. The method according to any one of the preceding claims, wherein the microorganism is cultured in a medium containing a sugar carbon source selected from the group consisting of arabinose, fructose, galactose, glucose, lactose, maltose, sucrose, xylose, and combinations thereof.

10. The method according to any one of the preceding claims, wherein the microorganism is cultured aerobically.

11. The method according to any one of the preceding claims, wherein the microorganism is cultured anaerobically.

12. The method according to any one of the preceding claims, further comprising the step of isolating the 1,2-propanediol formed.

13. The method according to any one of the preceding claims, wherein the microorganism is cultured under conditions favorable to the production of intracellular methylglyoxal.

14. The method according to any one of the preceding claims, wherein a recombinant microorganism further containing a recombinant methylglyoxal synthase gene is cultured.

15. The method according to any one of the preceding claims, wherein a recombinant microorganism further containing a recombinant pyridine nucleotide transferase gene is cultured.

16. A synthetic operon which enables the production of 1,2-propandiol in a microorganism transformed to contain the operon, the operon comprising one or more genes whose encoded gene products catalyze the reduction

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of methylglyoxal to 1,2-PD and a promoter sequence operationally linked to the one or more genes.

17. The synthetic operon of Claim 16, wherein the one or more genes are selected from the group consisting of an aldose reductase gene, a glycerol dehydrogenase gene, and combinations thereof.

18. The synthetic operon of Claim 16 or 17, further comprising one or more genes whose encoded gene products catalyze increased production of intracellular methylglyoxal.

19. The synthetic operon of any one of Claims 16, 17, or 18, comprising a methylglyoxal synthase gene.

20. A synthetic operon comprising at least one promoter sequence, a gene selected from the group consisting of an aldose reductase gene, a glycerol dehydrogenase gene, and combinations thereof; and a gene selected from the group consisting of a methylglyoxal synthase gene, a pyridine nucleotide transferase gene, and combinations thereof, wherein the genes are operationally linked to the at least one promoter.

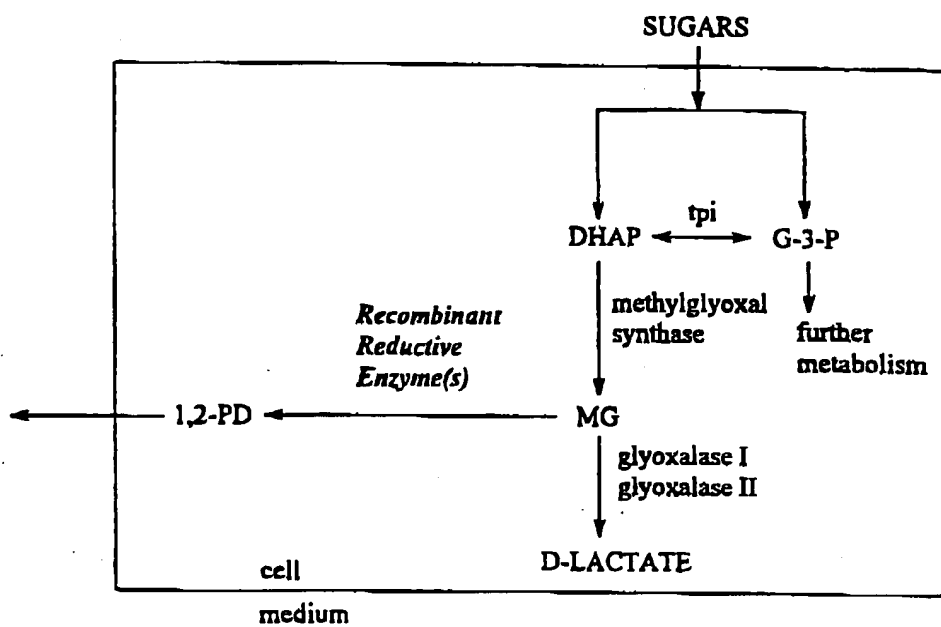
21. The synthetic operon of Claim 20, comprising SEQ. ID. NO: 3, SEQ. ID. NO: 6, and SEQ. ID. NO: 7.

22. The synthetic operon of Claim 20 or 21, comprising SEQ. ID. NO: 10 and SEQ. ID. NO: 6.

23. An *E. coli* transformed with a synthetic operon as recited in any one of Claims 16-22.

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**FIG. 1**

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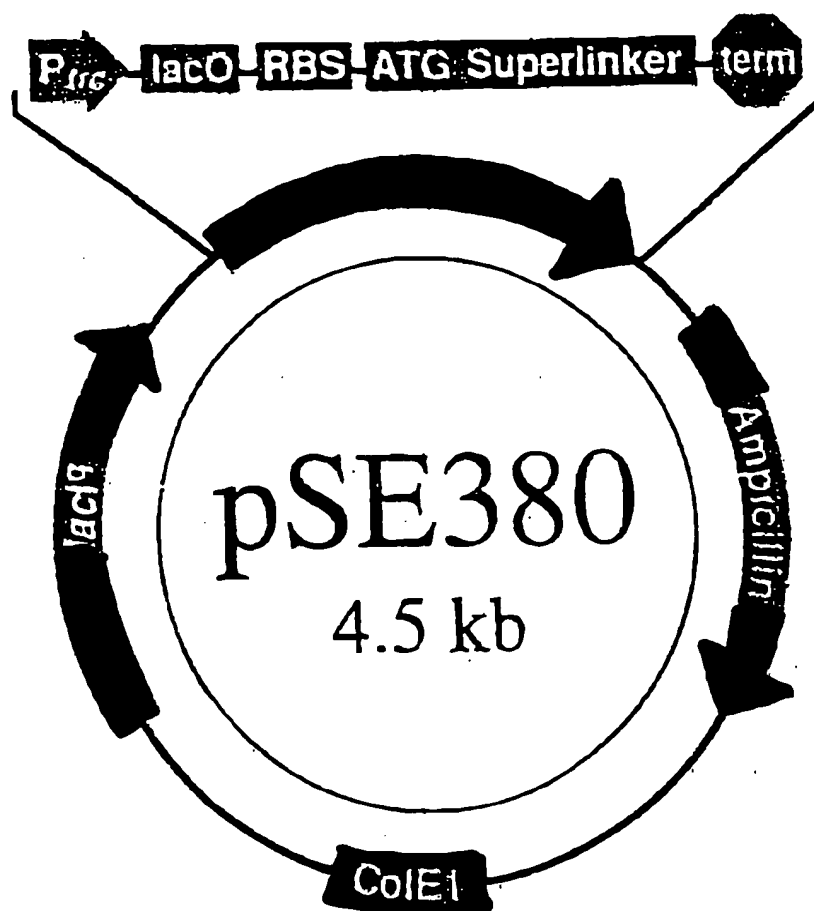


FIG. 2

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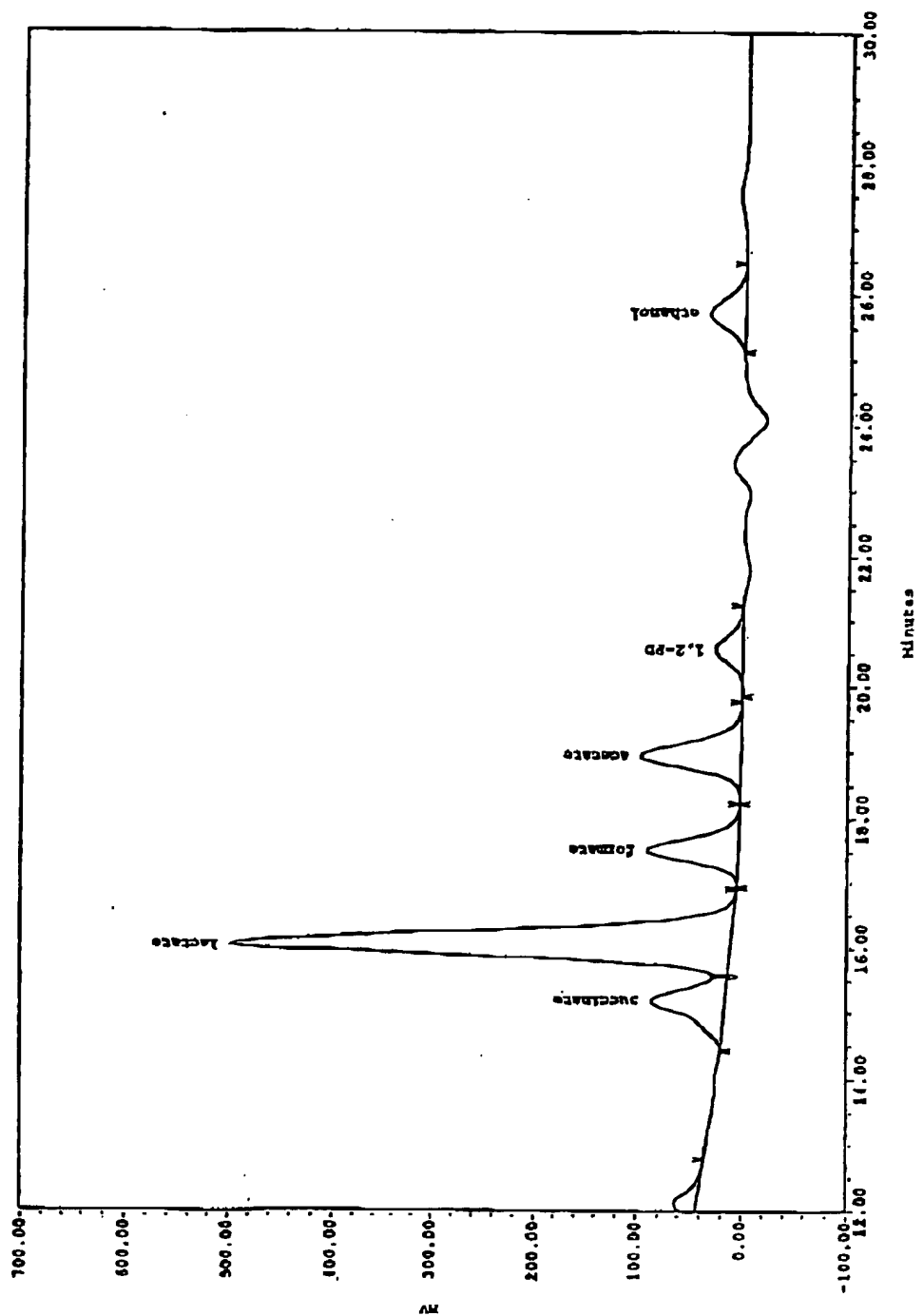


FIG. 3

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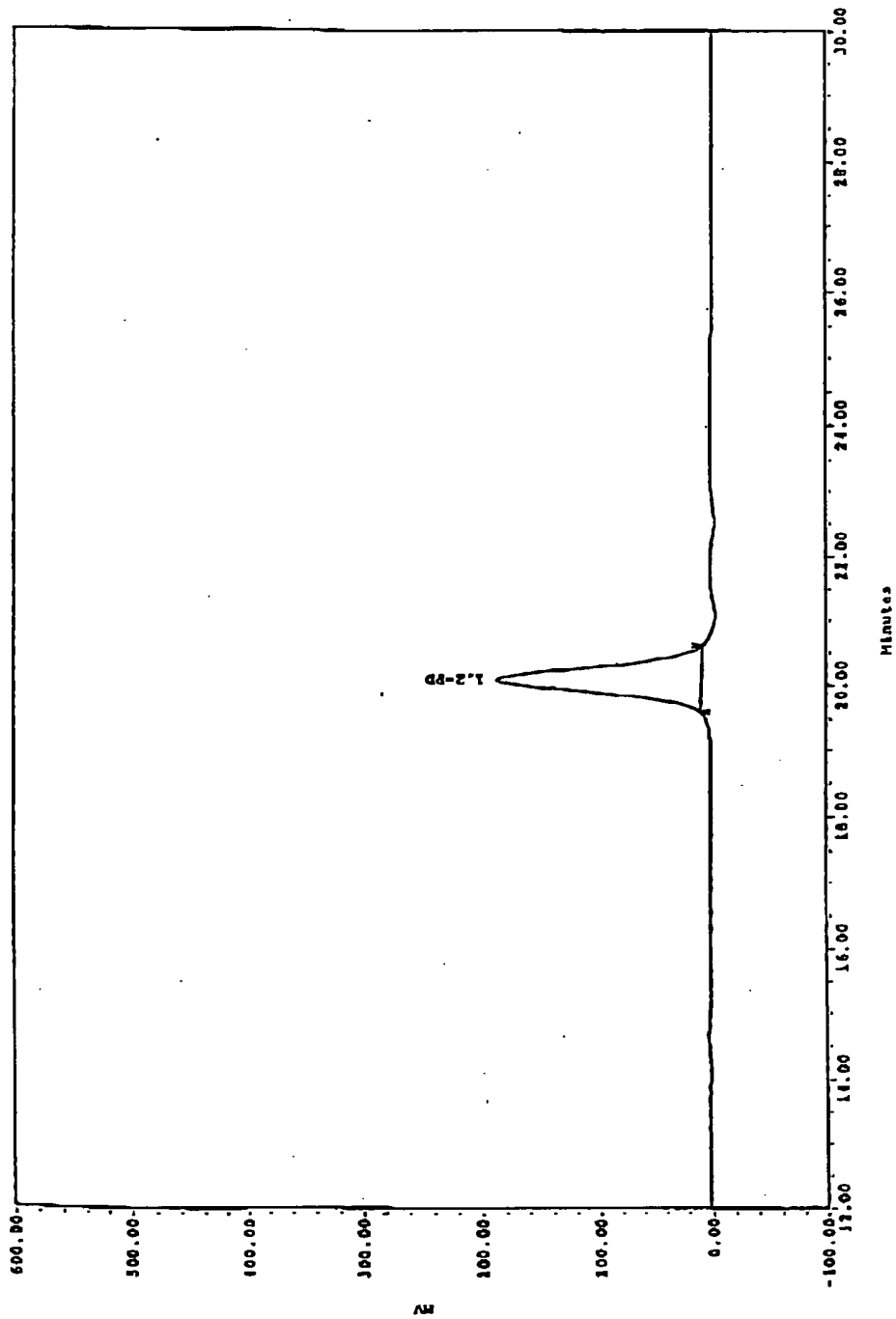


FIG. 4

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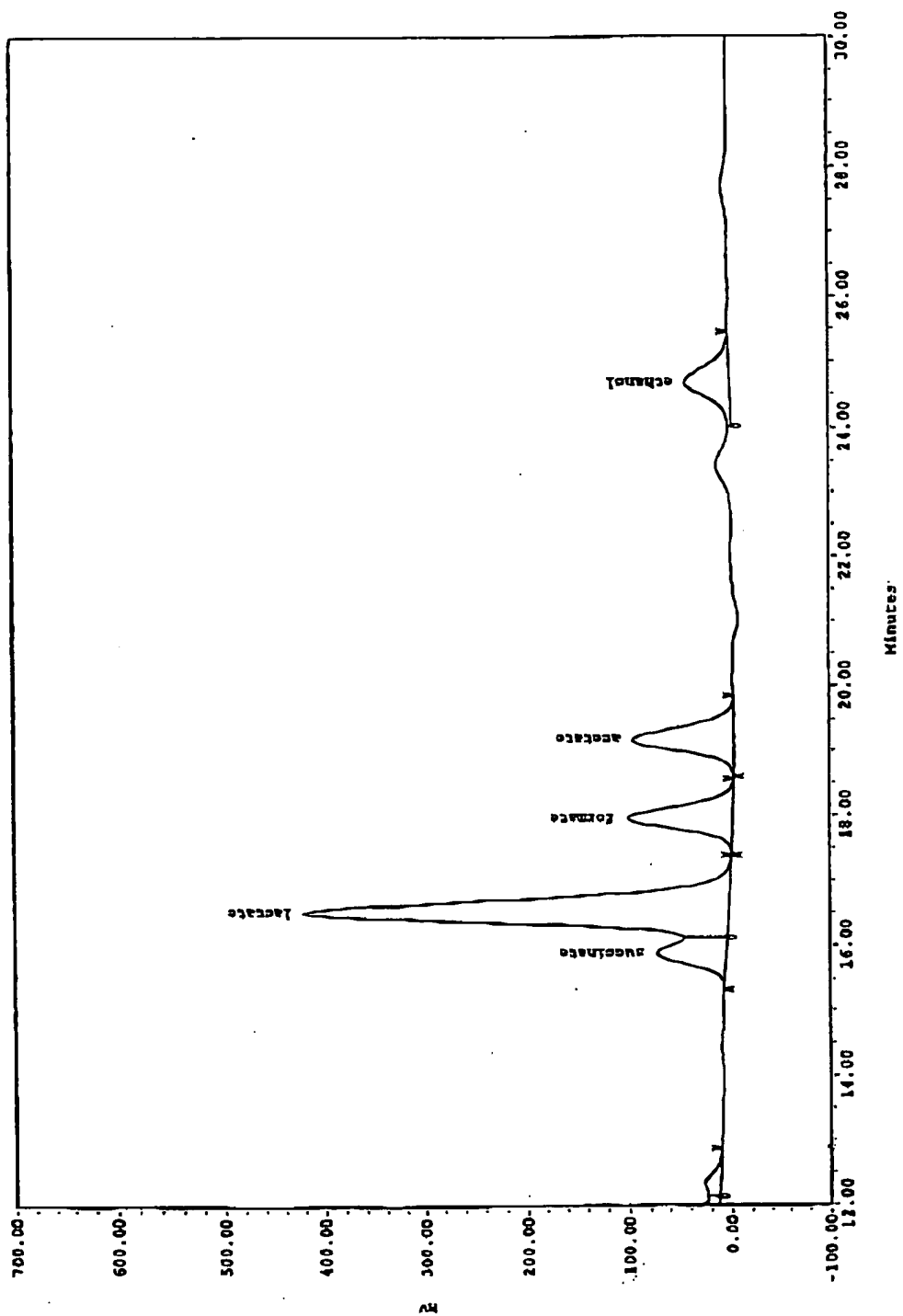


FIG. 5

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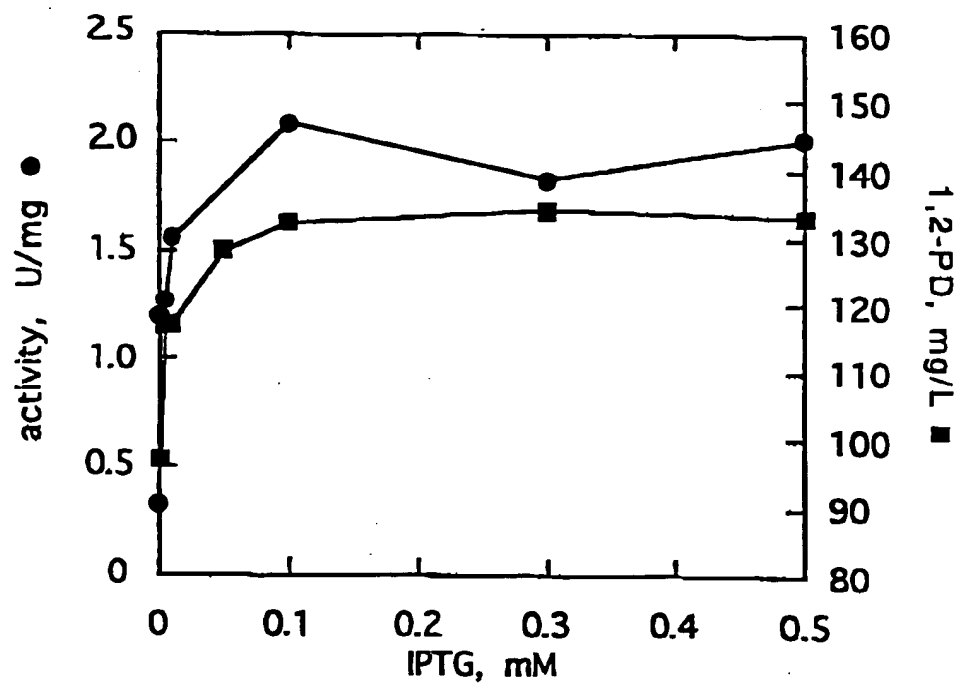


FIG. 6

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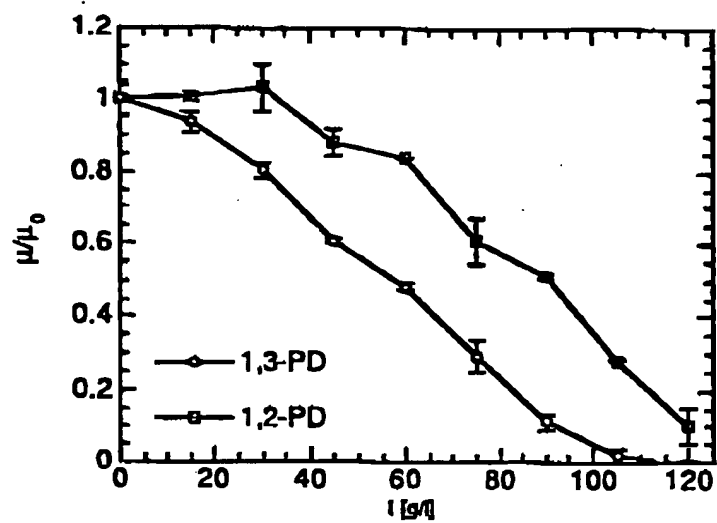


FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/03271

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 6	C12N15/53 C12P7/18 //(C12P7/18,C12R1:19)	C12N9/04 C12N1/21 C12N9/88
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC 6 C12P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	CAMERON D.C. ET AL.: "Metabolic engineering of propanediol pathways." BIOTECHNOLOGY PROGRESS, vol. 14, no. 1, 6 February 1998, pages 116-125, XP002067772 see abstract see figure 1 see page 120, column 2 - page 121, column 1 -/-	1-23
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "Δ" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
17 June 1998		30/06/1998
Name and mailing address of the ISA European Patent Office, P.O. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Lejeune, R

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page 1 of 3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/03271

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CAMERON D.C. & COONEY C.L.: "A novel fermentation: the production of R(-)-1,2-propanediol and acetol by <i>Clostridium thermosaccharolyticum</i> ." BIO/TECHNOLOGY, vol. 4, 1986, XP002067773 cited in the application see abstract see figure 3	1,9-12
X	OLD S.E. ET AL.: "In vitro expression of rat lens aldose reductase in <i>Escherichia coli</i> ." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 87, July 1990, pages 4942-4945, XP002067774 cited in the application see abstract	16,17,23
X	TRUNIGER V. & BOOS W.: "Mapping and cloning of <i>gldA</i> , the structural gene of the <i>Escherichia coli</i> glycerol dehydrogenase." JOURNAL OF BACTERIOLOGY, vol. 176, no. 6, March 1994, pages 1796-1800, XP002067775 see abstract	16,17,23
X	HILL P.W. ET AL.: "Bioenergetics and end-product regulation of <i>Clostridium thermosaccharolyticum</i> in response to nutrient limitation." BIOTECHNOLOGY AND BIOENGINEERING, vol. 43, 1993, pages 873-883, XP002067776 see figure 1 see figures 6-9	1,9-12
Y	TRAN-DIN K. & GOTTSCHALK G.: "Formation of D(-)-1,2-propanediol and D(-)-lactate from glucose by <i>Clostridium sphenoides</i> under phosphate limitation." ARCHIVES OF MICROBIOLOGY, vol. 142, 1985, pages 87-92, XP002067777 cited in the application see abstract see figure 2 see page 90, column 2, paragraph 4 - page 91, column 1, paragraph 2	1-7,9-13
Y		2-7,13

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INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 98/03271

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VANDER JAGT D.L. ET AL.: "Reduction of trioses by NADPH-dependent aldo-ket reductases." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 7, 5 March 1992, pages 4364-4369, XP002067778 see abstract see page 4367, column 2, scheme 1	1-7,9-13
Y	LEE L.G. & WHITESIDES G.M.: "Preparation of optically active 1,2-diols and alpha-hydroxy ketones using glycerol dehydrogenase as catalyst: limits to enzyme-catalyzed synthesis due to noncompetitive and mixed inhibition by product." JOURNAL OF ORGANIC CHEMISTRY, vol. 51, no. 1, 1986, pages 25-36, XP000602264 see abstract see table 1	1-7,9-13